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Phytochemical Constituents and Antioxidant Properties of Aqueous Leaf Extract of *Myrianthus arboreus*

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Abstract: This study was carried out to determine the phytochemical compositions and in vitro antioxidant properties of aqueous leaf extract of *Myrianthus arboreus* plant. The phytochemical analysis was determined using standard laboratory methods and the in vitro antioxidant properties assessed by the 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical scavenging and Reducing Power assay. The leaf extract showed the presence of saponins, tannins, alkaloids, flavonoids, glycosides and total phenol. These phytochemicals are in varying concentrations: saponins (2.05±0.02mg/g), flavonoids (0.33 ± 0.02mg/g), alkaloids (0.02±0.00mg/g), tannins (5.32 ± 0.01mg/g), total phenol (11.02 ± 1.00mg/g) and glycosides (0.48 ± 0.00mg/g). Also, the result of the antioxidant studies revealed that the percentage inhibition of *Myrianthus arboreus* aqueous leaf extract against free radical at different concentrations of 125µg/ml, 100µg/ml, 75µg/ml, 50µg/ml and 25µg/ml for both assays gave 74.4% and 64.5% respectively as compared to the standard (Ascorbic acid) values of 101.0% and 94.1%. Thus, the antioxidant activity of the extract was significant ($p<0.05$) for both assays when compared with the standard. However, the Reducing Power assay revealed increased significance. This implies that the inhibitory concentration (IC_{50}) of *Myrianthus arboreus* leaf extract is lower than the standard ascorbic acid and therefore suggests that it is a potent antioxidant capable of scavenging excess free radicals. In conclusion, it can be deduced that the phytochemical compositions especially the total phenols which are higher in concentration when compared with other phytochemicals are responsible for the antioxidant properties of the plant and can invariably serve as natural supplements of antioxidant in foods and pharmaceutical products.

Keywords: Antioxidant, Free Radicals, *Myrianthus arboreus* and Phytochemicals

1. Introduction

Reactive oxygen species (ROS) are generated by living organisms from normal cellular processes during energy production. These free radicals are beneficial in physiological processes at low moderate concentrations. However, at elevated concentrations it results in damaging effects on major cellular components such as DNA, proteins, lipids. This imbalance in antioxidants and oxidants lead to oxidative stress. In addition, the balance between antioxidants and ROS are disrupted as a result of either the depletion of antioxidants or accumulation of ROS [1, 2, 3]. The body generates energy by gradually oxidizing its food in a controlled manner and storing it in the form of chemical potential energy called adenosine triphosphate (ATP). This energy generation mechanism which is essential to life can also set the stage for cell damage. The oxidation of food liberates energy but also free electrons escaping the transport system. These unpaired electrons readily form free radical molecules which easily react with other molecules, setting off a chain reaction of free radical formation and thus giving rise to potential damage [4].

Furthermore, it has being discovered that major chronic diseases such as cancer, atherosclerosis, hypertension, ischemia and degenerative disorders like Alzheimer and Parkinson are linked to oxidative stress arising from these free radicals generated within the body [5].

Although, living organisms have coordinated systems (both enzymatic and non enzymatic) for antioxidants; capable of ameliorating the harmful effects of free radicals,

this however becomes difficult in diseased conditions. Thus, the need for supplementary antioxidants.

The plant *Myrianthus arboreus* belongs to the family of *Cecropiaceae*. It is a dioecious tropical tree which is up to 15 meters high with spreading branches from a short stem. The leaves are large, alternately shaped 5-7 digitated compound, coarsely toothed with hood-like edges; the central leaflet is about 25×9cm. It has stilt roots, trunk are short, diving into spreading crowns. Its young leaves are usually red in color. Its flowers are seen between January and April [6, 7].

(a)

(b)

Figure 1. *Pictures of Myrianthus arboreus with its flower radially arranged leaf vein.*

The various parts of the plant have numerous medicinal values. The leaves, sap and bark are potent antimicrobial and analgesic agents. The plant possesses macromolecules like carbohydrates, minerals, oil, proteins and vitamins and medicinal importance for the control of diuretic and hypertensive complications [8]. However, the universe especially Africa, including Nigeria and Ghana is endowed with vast varieties of underutilized species of vegetables which are found in localized regions and are consumed by rural populace for various perceived health benefits with little or no scientific investigations. Among such vegetables is the *Myrianthus arboreus* plant. They are known for their protective roles against free radicals; because the leaves contain vitamin C which makes them good source of antioxidants [9, 10].

This paper therefore focuses on determining the phytochemical compositions and in vitro antioxidant properties of the aqueous leaf extract of *Myrianthus arboreus*.

2. Materials and Methods

The fresh leaves of *Myriathus arboreus* were obtained from Ogbor-Hill, Abia state, Nigeria. The fresh and healthy leaves were identified in the Department of Biological Sciences (Botany sector), Kogi State University, Anyigba.

2.1. Extraction and Preparation of Aqueous Leaf Extract

The leaves of *Myrianthus arboreus* were air dried in Biochemistry Laboratory under room temperature and pulverized to powder using a blender. 108.73g of the powder of *Myrianthus arboreus* leaves was macerated in 1.2 litres of distilled water. It was stirred to obtain a homogenous mixture and allowed to stand in a dark room for 24 hours. After 24 hours, the mixture was filtered usinga vacuum extractor and the extract was concentrated by heat evaporation at a temperature below 80°C. The concentrated crude extract was stored in a refrigerator to avoid spoilage before being used for the analysis.

2.2. Phytochemical Analyses

Both qualitative and quantitative analyses were carried out on the aqueous leaf extract of *Myrianthus arboreus* using standard phytochemical procedures by different authors.

2.2.1. Qualitative Phytochemical Analysis

Qualitative analysis was carried out to determine the presence of phytochemical constituents in the aqueous leaf extract of *M. arboreus* using standard phytochemical procedures by [11, 12, 13].

2.2.2. Quantitative Phytochemical Analysis

Phytochemicals in the aqueous leaf extracts of *Myrianthus arboreus* were quantified using standard method by [14] for determination of saponin, tannins [15], alkaloids [16], flavonoids, total phenol and glycosides by the method of [17].

2.3. Determination of the Antioxidant Properties of Aqueous Leaf Extract of Myrianthus arboreus

The antioxidant properties were assessed by the methods described by [18].

2.3.1. DPPH Free Radical Scavenging Activity

The DPPH solution was prepared by dissolving 3 mg of

DPPH in 50 ml of methanol. To 1 ml of various concentrations (0.02, 0.04, 0.06, 0.08, 0.10 mg/ml) of the extract of *Myrianthus arboreus*, 2 ml of DPPH solution (0.1mM) was added. An equal amount of methanol and DPPH was used as control. Each mixture was shaken vigorously and left to stand in the dark for 30 minutes. The absorbances of the resulting solutions were measured using a spectrophotometer in triplicates at 520 nm and the percentage scavenging activity was calculated using the formula below.

$$\% \text{ scavenging activity} = \frac{1 - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100 \quad (1)$$

Similar procedure was repeated for the standard Ascorbic acid to obtain the percentage inhibition.

2.3.2. Reducing Power Assay

The reducing power ability of the leaf extract was evaluated by the method described by [19]. Different concentrations of the extract (25, 50, 75, 100 and 125 µg/ml) were prepared in methanol. Sample solution (03ml: 100/gml^{-1}) was mixed with reagent solution (3ml; 0.6m sulphuric acid, 2.8 mM sodium phosphate and 4mM Ammonium molybdate). A blank composed of 3 ml of reagent solution and methanol was also prepared. All test tubes were capped and incubated in boiling water bath at 95°C for 90 minutes. Absorbances of samples were read against blank at 695 nm. The antioxidant activity of sample was expressed as mg ascorbic acid equivalent.

2.4. Statistical Analysis

To test for level of significance, raw data from both the phytochemical analysis and in vitro antioxidant studies were collected in triplicates and values expressed as Mean±SD and then subjected to statistical software, SPSS version 17. Differences between groups are considered significant at $p< 0.05$.

3. Results

3.1. Results of Phytochemical Analysis

Tables 1 and 2 showed the qualitative and quantitative phytochemical analyses of *Myrianthus arboreous* aqueous leaf extract. The extract shows the presence of phytochemicals at varying amount.

Table 1. *Qualitative Phytochemical Analysis of aqueous leaf Extract of Myrianthus arboreus.*

Phytochemicals	Intensity
Alkaloids	+
Saponins	+
Tannis	++
Flavonoids	+
Total phenol	++
Glycosides	+

Key: ++ = high concentration
+ = Low concentration

Table 2. *Quantitative Phytochemical Analysis of aqueous leaf Extract of Myrianthus arboreus.*

Phytochemicals	Concentration (mg/g)
Glycosides	0.48 ± 0.00
Flavonoids	0.33 ± 0.02
Saponins	2.05 ± 0.02
Tanins	5.32 ± 0.01
Total Phenols	11.02 ± 1.00
Alkaloids	0.02±0.00

Values are expressed as mean ± standard deviation, n = 3

3.2. In Vitro Antioxidant Activity

Tables 3 and 4 are results of the DPPH free radical scavenging assay of aqueous leaf extract of *Myrianthus arboreus* and the standard (Ascorbic acid) while Tables 5 and 6 are for the Reducing Power assay at different concentrations (125µg/ml, 100µg/ml, 75µg/ml, 50µg/ml and 25µg/ml) respectively.

Table 3. *DPPH Free Radical Scavenging Activity of Myrianthus arboreus Aqueous Leaf Extract (Absorbance of Control: 0.705).*

Concentration of extract(µg/ml)	Absorbance	%inhibition	IC_{50}
125	0.180	74.5	
100	0.235	66.7	
75	0.342	51.5	70.4
50	0.401	43.1	
25	0.526	25.4	

Values are expressed as mean ± standard deviation, n = 3

Table 4. *DPPH Free Radical Scavenging Activity of Ascorbic acid standard (Absorbance of Control: 0.705).*

Concentration of extract(µg/ml)	Absorbance	%inhibition	IC_{50}
125	0.290	58.9	
100	0.351	50.2	
75	0.421	40.3	101.0
50	0.501	28.9	
25	0.621	11.9	

Values are expressed as mean ± standard deviation, n = 3

Table 5. *Reducing Power of Myrianthus arboreus aqueous leaf extract (Absorbance of Control:0.710).*

Concentration of extract(µg/ml)	Absorbance	%inhibition	IC_{50}
125	0.120	83.1	
100	0.201	71.7	
75	0.312	56.1	64.5
50	0.408	42.5	
25	0.521	26.6	

Values are expressed as mean ± standard deviation, n = 3

Table 6. *Reducing Power of Ascorbic Acid Standard (Absorbance of Control: 0.710).*

Concentration of extract(µg/ml)	Absorbance	%inhibition	IC_{50}
125	0.210	70.4	
100	0.350	50.7	
75	0.460	35.2	94.1
50	0.520	26.8	
25	0.610	14.1	

Values are expressed as mean ± standard deviation, n = 3

In addition, Figure 2 and Figure 3 are linear representations of the antioxidant assay for both DPPH scavenging assay, Reducing power assay and the Standard (Ascorbic acid). By implication, the extract straight line graph is more linear when compared to the ascorbic acid, suggesting that it is a more potent antioxidant than the known age-long ascorbic acid.

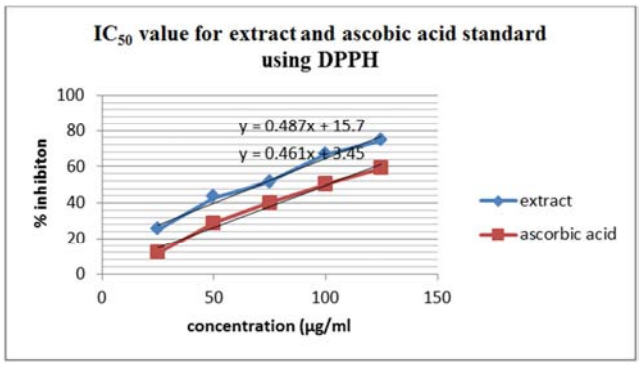

Figure 2. *Graph showing IC_{50} value of extract and ascorbic acid standard using DPPH scavenging assay.*

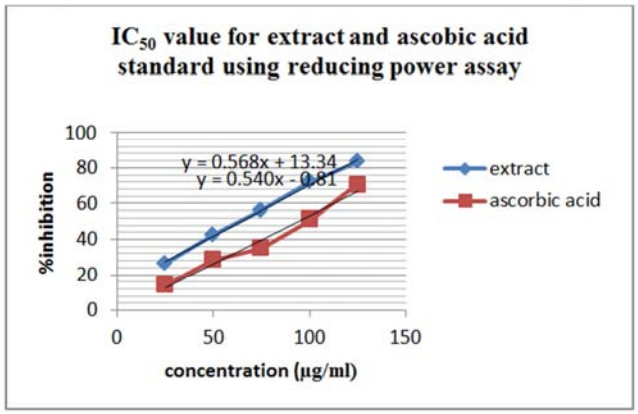

Figure 3. *Graph showing IC_{50} value of extract and ascorbic acid standard using reducing power assay.*

4. Discussion

The aqueous leaf extract of *Myrianthus arboreous* plant was screened for phytochemicals qualitatively and quantitatively (Tables 1 and 2). This is significant because, the constituents in plants form the basis of chemical synthesis of natural products and subsequent investigations for their biological activities [20, 21]. The phytochemicals are in varying concentrations, with total phenols having the highest concentration which could be indicative of antioxidant and other medicinal properties of the plant [22]. Thus supporting the studies reported by [23] that nearly all plants have one or more phytochemicals resident in their leaf, stem, barks and roots which makes them unique for the treatment of various diseases. Although *Myrianthus arboreus* has the highest concentration of total phenol, there are however significant differences in the values recorded for other leafy vegetables. The concentration in this study is higher than those reported by [24] for *Telfaria accidentalis* (1.13 ± 0.13mg/g), *Venonia amydaline* (1.10 ± 0.6mg/g), *Cnidoscolus kaconitifolius* (1.16 ± 0.16), *Hibiscus asper* (1.17 ± 0.09mg/g), while *Gongonemalatifolium* has total phenol concentration of 12.0 ± 0.00 [25] which is close to *Myrianthus arboreus.* Similarly, phenolic compound have hypo-cholesterolemic properties as reported by [26]. Therefore, it may play a role in prevention of chronic degenerative disease such as cancer and cardiovascular diseases and confer some chemo-protection against these disorders to users of *Myrianthus arboreus* as this plant is more effective antioxidants than vitamins E and C [27].

Also, the in vitro antioxidant activity showed an IC_{50} for *M. arboreus* aqueous leaf extract using DPPH scavenging assay and standard at different concentrations (125µg/ml, 100µg/ml, 75µg/ml, 50µg/ml and 25µg/ml) as 74.4% and 101.0% (Tables 3, 4 and Figure 2). On the other hand, (Tables 5, 6 and Figure 3) shows results for Reducing power assay and standard, at the same concentrations as in DPPH. The $IC_{50}$of the extract and standard are 64.5% and 94.1% respectively for both assays. Comparing both results, it revealed that the extract of *Myrianthus arboreus* has lower IC_{50} values when compared to the standard (ascorbic acid) suggesting the potency of the extract. In relation to this, it has being reported that the lower the IC_{50} of a sample, the more effective or potent antioxidant it is. Therefore, it can be deduced that *Myrianthus arboreus* plant is potent and a good antioxidant. It also implies that *Myrianthus arboreus* leaf extract contains compounds that are capable of donating hydrogen to free radical in order to remove odd electron which are responsible for radical reactivity [26]. These compounds include the phytochemicals present in the extract such as the total phenol or flavonoid content. It further shows that the extract is a potent antioxidant and beneficial in the treatment of diseases such cardiovascular, cancers and other neurodegenerative disorders resulting from oxidative stress.

5. Conclusion

In conclusion, the aqueous leaf extract of *Myrianthus arboreus* contain phytochemicals especially total phenols in high concentration which is specific to antioxidants among others. This could be indicative of the antioxidant properties observed in the plant. It can therefore be recommended as antioxidant supplements in food and pharmaceutical products as their bioactive composition are capable of scavenging free radicals and thus ameliorating the damaging effects of these reactive species on cellular components and could possibly be effective in managing free radical linked disorders.

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