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# IN VITRO ANTIOXIDANT ACTIVITY, MINERALS AND PHYTOCHEMICAL COMPOSITION OF *Amaranthus hybridus* LEAVES

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## ABSTRACT
*Amaranthus hybridus* constitutes a major part of the diet of the people in the middle and southern parts of Nigeria, where they are mostly used in soups. This study was carried out to screen for bioactive agents, minerals using standard procedures as well as *in vitro* antioxidant activity using reducing power and DPPH scavenging assays. The result of screened phytochemicals showed the presence of flavonoids, alkaloids, tannins, saponins while reducing power and DPPH scavenging activity showed a concentration dependent increase as the concentration increased from 0.2mg/ml to 1.0mg/ml. Ascorbic acid content was found to be 154 ± 8.76mg/100g. Of the minerals in mg/100g, calcium was higher in concentration (320.60 ± 1.51) while chromium was least in concentration (0.68 ± 0.20). Other minerals present were magnesium (153.60 ± 1.13), potassium (132.40 ± 1.11), sodium (69.05 ± 0.82), iron (36.79 ± 0.70), zinc (4.07 ± 0.32), copper (1.81 ± 0.20) and manganese (1.21 ± 0.23). The presence of secondary metabolites such as flavonoids as well as minerals, ascorbic acid and *in vitro* antioxidants activities in *Amaranthus hybridus* leaves contributes to their medicinal and health benefits.

**Keywords:** *Amaranthus hybridus,* Antioxidants, Minerals, Phytochemicals

## INTRODUCTION
*Amaranthus hybridus* (Amaranthaceae) commonly known as smooth pigweed is an erect branched annual herb distributed throughout tropical and temperate regions as a common weed in the fields and wastelands (Krochmal *et al.*, 1973). It is an annual herbaceous plant in Nigeria often combined with condiments for soup preparation (Oke, 1983; Mepha *et al.,* 2007). In Congo, it is eaten as spinach or green vegetables (Dhellot *et al.,* 2006). In Mozambique and in West Africa, the leaves boiled and mixed with groundnut sauce are eaten as salad (Oliveria and DeCarvalho, 1975; Martin and Telek, 1979). *A. hybridus* has been used traditionally for the treatment of liver infections and knee pain and for its laxative, diuretic, and cicatrisation properties (Nacoulma, 1996); treating diarrhoea, dysentery, ulcers and hemorrhage of the bowel due to its astringent property (Krochmal and Krochmal, 1973). The leaves possess antibacterial effect (Cyrus *et al.,* 2008), cleansing effect and also help to reduce

tissue swelling (Bhattacharjee, 2004). This work was therefore aimed at documenting the minerals, phyto-agents and *in vitro* antioxidant potentials of *A. hybridus* leaf in order to provide information on its usefulness as an edible vegetable.

## MATERIALS AND METHOD

### *Preparation of A. Hybridus Leaves*

The leaves of *A. hybridus* were bought from Santana market, Sapele road, Benin City, Edo state, Nigeria. The leaves were identified by a Botanist and thereafter brought to Department of Biochemistry Laboratory, Benson Idahosa University, Nigeria. The leaves were rinsed and dried for three weeks at ambient temperature after which they were milled into fine powder in a manual grinder and preserved in an air tight container.

### *Phytochemical Screening*

The powdered leaves were tested for the presence of phytochemicals as previously described by Trease and Evans (1985), Usunobun *et al.,* (2015) and Usunobun and Okolie, (2016) as follows:

**(i)** ***Test for Alkaloids:*** Powdered leaf sample (0.2 g) was boiled with 5 ml of 2% hydrochloric acid on a steam bath for 5 minutes. The mixture was allowed to cool and filtered and the filtrate was divided equally into 2 test tubes and labeled A and B. Aliquots (1 ml portions) of the filtrate were treated with 2 drops of either Dragendroff`s reagent or Mayer`s reagent. With Dragendroff's reagent a red precipitate was observed while Mayer`s reagent gave a creamy white precipitate. Both indicated the presence of alkaloids.

**(ii)** ***Test for Saponins:*** Leaf sample (0.5 g) was shaken with water in a test tube and warmed in a water bath. The persistence of froth indicates the presence of saponins.

**(iii)** ***Test for Anthraquinones:*** Leaf sample (0.5 g) was shaken with 10 ml of benzene and the suspension was filtered. Ammonia solution (0.5 ml, 10%) was added to the filtrate and the mixture was shaken well. The presence of a violet colour (in the layer phase) indicated the presence of the anthraquinones.

**(iv)** ***Test for Flavonoids:*** Leaf sample (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicated the presence of flavanoids.

**(v)** ***Test for Tannins:*** Ground leaf sample (0.5 g) was stirred with 10 ml of distilled water. The suspension was filtered and 0.1% ferric chloride reagent was added to the filtrate. A blue black colouration indicated the presence of tannins.

**(vi)** ***Test for Glycosides:*** Leaf sample (2 g) was mixed with 30 ml of distilled water, heated for 5 minutes in a water bath and filtered. The filtrate (5 ml) was added to 5 ml of Fehling solution A and Fehling solution B, mixed well and boiled for 4 min. The conversion of blue colouration into brick-red precipitate indicated the presence of glycosides.

### *Mineral Analysis of A. hybridus Leaves*

Mineral analysis was carried out using atomic absorption spectrophotometer (Usunobun and Okolie, 2015a, b). Briefly, an acid digest of *A. hybridus* powdered leaf was

prepared by oxidizing 0.2g of the sample with conc. HCl/nitric acid followed by kjeldahl heating at 70°C until the brown fumes disappeared. The digest was diluted with distilled water and heated again (until colourless solution is obtained) and thereafter filtered through Whatmann No. 1 filter paper. The filtrate was made up to 100 ml with distilled water and aliquots were used for mineral analysis. The blank and working standards were first run followed by the samples. Analysis was done in duplicates and the result reported as mg/100g of sample.

***Determination of Ascorbic Acid Content***

Ascorbic acid content was determined titrimetrically using the 2, 6-dichloroindophenol-indophenol method (Nielsen, 2010) as follows: Standard indophenol solution was prepared by dissolving 0.05g of 2,6-dichloroindophenol in 100 ml distilled water and filtered. Ascorbic acid standard was prepared by dissolving 0.053g of ascorbic acid in 90ml of 20% metaphosphoric acid and making up to 100 ml with distilled water. an aliquot (10 ml) of the solution was pipetted into a small conical flask and titrated against the standard indophenol solution until a faint pink colour persisted for 15 s. The titre value was noted. Powdered *A. hybridus* leaf (0.5 g) was mixed with 20ml distilled water and filtered. Two ml of the filtrate was pipetted into a conical flask and 5ml of 20% metaphosphoric acid was added and the mixture was made up to 10ml with distilled water. The solution was then titrated against the indophenol solution until a faint pink colour persisted for 15 s. The ascorbic acid content of the samples was calculated using the formula:

$$\text{Ascorbic acid in mg/100g} = \frac{\text{Titre value x dye factor x total volume of extract x 100}}{\text{volume of aliquot x weight of sample}}$$

***Test for Reducing Power***

The reducing power of *A. hybridus* leaves was carried out using the reducing power method as described by Aiyegoro and Okoh (2010). A mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of stock *A. hybridus* leaves filtrate (0.2–1.0 mg/ml) prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 ml of TCA (10% w/v), followed by centrifugation at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was measured at 700 nm against reagent blank sample. Increased absorbance of the reaction mixture indicates higher reducing power of *A. hybridus* leaves.

***Test for DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrazyl) Radical Scavenging Activity***

The DPPH method according to Liyana-Pathiana and Shahidi (2005) was used for the determination of DPPH radical scavenging activity of *A. hybridus* leaves as follows: DPPH (1 ml, 0.135 mM) prepared in methanol was mixed with 1.0 ml of stock *A. hybridus* leaf filtrate ranging in concentration from 0.2 to 1.0 mg/ml. The reaction mixture was then vortexed thoroughly

and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. The scavenging ability was calculated using the equation:

DPPH scavenging activity (%) = [(Abs $_{control}$ − Abs $_{sample}$)]/ (Abs $_{control}$)] × 100,

Where: Abs $_{control}$ is the absorbance of DPPH + methanol and Abs $_{sample}$ is the absorbance of DPPH radical + sample.

## RESULTS

The results of qualitative phytochemical analysis of of *A. hybridus* leaves are given in Table 1. Results indicate the presence of several phyto-components including alkaloids, flavonoids, saponins, tannins and the absence of glycosides, anthraquinones, phlobatannins. The result of Ascorbic acid showed *A. hybridus* leaf to contain 154 ± 8.76 mg/100g.

Table 1: The Qualitative phytochemical screening of *A. hybridus*

| Phytochemicals | *A. hybridus* |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Glycosides | - |
| Tannins | + |
| Anthraquinones | - |
| Phlobatannins | - |
| Saponins | + |

Where **+** = present, **-** = absent

The result of the minerals analysis carried out on *A. hybridus* leaves as shown in Table 2 revealed the presence of calcium, magnesium, potassium, sodium, phosphorus, iron, zinc, copper, manganese, and chromium in different concentrations. Calcium was found present in higher concentration (320.60 mg/100 g). Others includes magnesium (153.60 mg/100 g), potassium (132.40 mg/100 g), sodium (69.05 mg/100 g), iron (36.79 mg/100 g), zinc (4.07 mg/100 g), copper (1.81 mg/100 g), manganese (1.21 mg/100 g), chromium (0.68 mg/100 g).

Table 2: Mineral analysis of *A. hybridus* leaves

| Minerals (mg/100 g) | *A. hybridus* |
|---|---|
| Calcium | 320.60±1.51 |
| Magnesium | 153.60±1.13 |
| Potassium | 132.40±1.11 |
| Sodium | 69.05±0.82 |
| Phosphorus | 159.51±1.36 |
| Iron | 36.79±0.70 |
| Zinc | 4.07±0.32 |
| Copper | 1.81±0.20 |
| Manganese | 1.21±0.23 |
| Chromium | 0.68±0.20 |

Values are means ± SD for 2 determinations

The result of reducing power of *A. hybridus* as shown in figure 1 revealed that at higher concentrations, there is an increase in absorbance of reaction mixture, indicating reducing power in the medicinal plants.

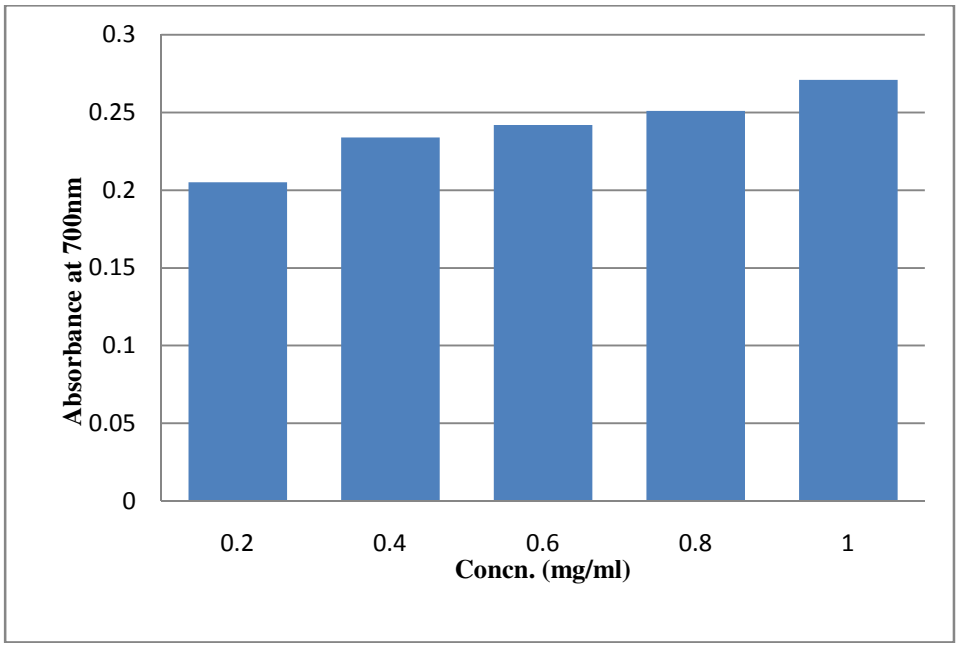


Figure 1: Reducing power of *A. hybridus* leaves

The result of DPPH radical scavenging of *A. hybridus* as shown in figure 2 revealed increasing % inhibition of DPPH radical by *A. hybridus* as concentration increases from 0.2 to 1.0mg/ml.

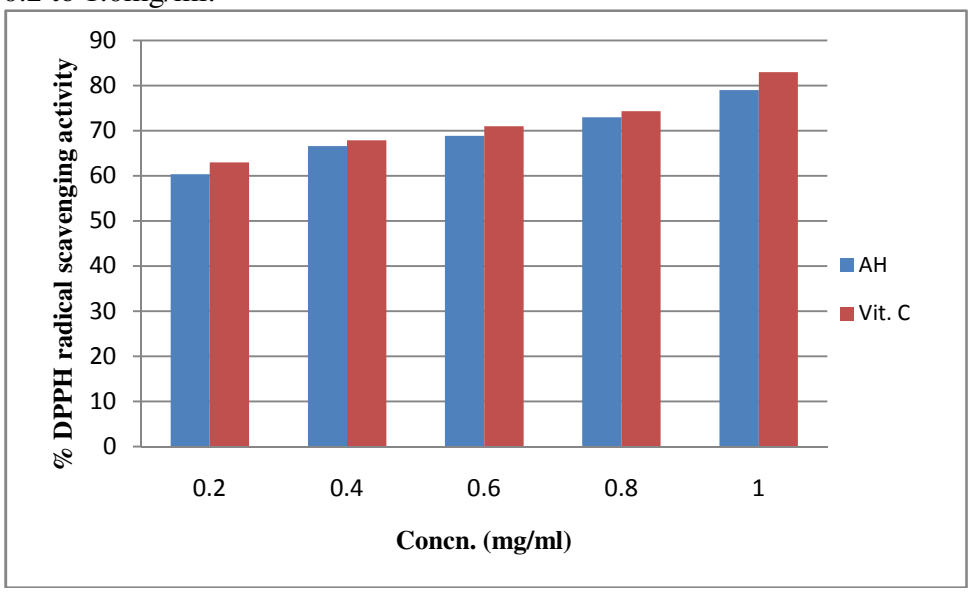


Figure 2: DPPH radical scavenging activity of *A. hybridus* (AH) leaves

## DISCUSSION

Minerals are required for normal growth, activities of muscles and skeletal development, regulation of acid-base balance, chemical reaction in the body and intestinal absorption, cellular activity and oxygen transport, fluid balance and nerve transmission etc.

Potassium content of *A. hybridus* (132.40 mg/100 g) compared favourably with values of 128.33 mg/100 g for *Celosia argentea* (Usunobun and Ekpemupolo, 2016) but high when compared to 96.91 mg/100 g for *Chromolaena odorata* (Usunobun and Ewere, 2016), 109.60 mg/100 g for *Ocimum gratissimum* (Usunobun and Uwadiae, 2016), 104.85 mg/100 g for *Pterocarpus mildbraedii* (Usunobun and Igwe, 2016b), 87.22mg/100g for *Solanum macrocarpon* (Usunobun and Igwe, 2016a), 36.31 mg/100 g for *Annona muricata* and 62.79 mg/100 g for *Vernonia amygdalina* (Usunobun and Okolie, 2015a, b). Potassium helps in the proper function of the brain as well as nerves, thereby preventing stroke. It plays part in acid-base and water regulation in the blood and tissues (Fakankun *et al.,* 2013). In addition to its contribution to the electrolytes and function of the nerves in the human body, potassium has been linked to bone health and osteoporosis prevention according to studies of intake of 2500 mg/day done in United Kingdom, United States and Australia ((Fakankun *et al.,* 2013, Susan and Lanham, 2008).

Calcium content of *A. hybridus* (320.60 mg/100 g) compared favourably with values of 295 mg/100 g for *C. argentea* (Usunobun and Ekpemupolo, 2016), high compared to 256.60 mg/100 g for *S. macrocarpon* (Usunobun and Igwe, 2016a), 128.30 mg/100 g for *P. mildbraedii* (Usunobun and Igwe, 2016b) and low when compared to 487.40 mg/100 g for *C. odorata* (Usunobun and Ewere, 2016), 436 mg/100 g for *O. gratissimum* (Usunobun and Uwadiae, 2016), 1118.30 mg/100 g for *A. muricata* and 1264.18 mg/100 g for *V. amygdalina* (Usunobun and Okolie, 2015a, b). Calcium is necessary for the coagulation of blood, the proper functioning of the heart and nervous system and normal contraction of muscles. Its most important function is to aid in the formation of bones and teeth (Ayoola, 2012).

Sodium content of *A. hybridus* (69.05 mg/100 g) compared favourably with values of 60.30 mg/100 g for *P. mildbraedii* (Usunobun and Igwe, 2016b), 69.49 mg/100 g for *A. muricata* leaves (Usunobun and Okolie, 2015a) and 71.32 mg/100 g for *C. argentea* (Usunobun and Ekpemupolo, 2016) but was high compared to 44.22 mg/100 g for *C. odorata* (Usunobun and Ewere, 2016), 48.95 mg/100 g for *O. gratissimum* (Usunobun and Uwadiae, 2016), 48.31 mg/100 g for *V. amygdalina* (Usunobun and Okolie, 2015b) and 32.51mg/100g for *S. macrocarpon* (Usunobun and Igwe, 2016a). Sodium and potassium are important intracellular and extracellular cations respectively. Low sodium diet has been reported to be beneficial in the prevention of high blood pressure and high potassium has been reported to have a protective effect against excessive sodium intake (Lichtenstein *et al.,* 2006).

Zinc content of *A. hybridus* (4.07 mg/100 g) compared favourably with values of 3.77 mg/100 g for *C. odorata* (Usunobun and Ewere, 2016), 3.30 mg/100 g for *O. gratissimum* (Usunobun and Uwadiae, 2016), 2.51 mg/100 g for *P. mildbraedii* (Usunobun and Igwe, 2016b) and 5.42 mg/100 g for *C. argentea* (Usunobun and Ekpemupolo, 2016) but was high compared to 0.83 mg/100 g for *A. muricata* (Usunobun

and Okolie, 2015a), 1.42 mg/100 g for *V. amygdalina* (Usunobun and Okolie, 2015b) and 1.41 mg/100 g for *S. macrocarpon* (Usunobun and Igwe, 2016a). Zinc plays important roles in growth and development, immune response, neurological function, and reproduction. It plays an important role in the structure of proteins and cell membranes. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function (O'Dell, 2000).

Copper content of *A. hybridus* (1.81 mg/100 g) compared favorably with 0.95 mg/100 g for *P. mildbraedii* (Usunobun and Igwe, 2016b), 2.18 mg/100 g for *C. argentea* (Usunobun and Ekpemupolo, 2016), 1.95 mg/100 g for *V. amygdalina*, 1.42 mg/100 g for *A. muricata* leaves (Usunobun and Okolie, 2015a, b), 1.35 mg/100 g for *O. gratissimum* (Usunobun and Uwadiae, 2016) and 1.41 mg/100 g for *C. odorata* (Usunobun and Ewere, 2016). Copper provides the catalytic activity for the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD).

Iron content of *A. hybridus* (36.79 mg/100 g) compared favorably with 31.41 mg/100 g for *S. macrocarpon* (Usunobun and Igwe, 2016a), 32.20 mg/100 g for *V. amygdalina* (Usunobun and Okolie, 2015b) and 35.16 mg/100 g for *C. argentea* (Usunobun and Ekpemupolo, 2016) but high when compared to 9.48 mg/100 g for *O. gratissimum* (Usunobun and Uwadiae, 2016), 14.64 mg/100 g for *P. mildbraedii* (Usunobun and Igwe, 2016b), 13.95 mg/100 g for *A. muricata* (Usunobun and Okolie, 2015a) and low when compared to 67.71 mg/100 g for *C. Odorata* (Usunobun and Ewere, 2016). Iron plays important role in building up of red blood cells essential for haemoglobin formation, the oxygen carrying pigment in red blood cells. Iron is used against anaemia, tuberculosis and growth disorder (Claude and Paule, 1979). Iron is an energizer but excess can cause fatigue but we hardly have excess if taken from natural source (Gbolahan, 2001).

Magnesium content of *A. hybridus* (153.60 mg/100 g) is high when compared to 77.80 mg/100 g for *P. mildbraedii* (Usunobun and Igwe, 2016b), 116.70 mg/100 g for *C. odorata* (Usunobun and Ewere, 2016), 122.50 mg/100 g for *C. argentea* (Usunobun and Ekpemupolo, 2016) and 81.69 mg/100 g for *S. macrocarpon* (Usunobun and Igwe, 2016a) but low when compared to 961.90 mg/100 g for *A. muricata* (Usunobun and Okolie, 2015a), 186.71 mg/100 g for *O. gratissimum* (Usunobun and Uwadiae, 2016), and 681.36mg/100g of *Vernonia amygdalina* (Usunobun and Okolie, 2015b). Magnesium plays some roles in human health, namely, production and energy transport, contraction and relaxation of muscles, synthesis of protein and function of enzymes (Fakankun *et al.,* 2013).

Chromium content of *A. hybridus* (0.68 mg/100 g) compared favorably with 0.89 mg/100 g for *O. gratissimum* (Usunobun and Uwadiae, 2016), 0.79 mg/100 g for *P. mildbraedii* (Usunobun and Igwe, 2016b), 0.37 mg/100 g for *A. muricata* (Usunobun and Okolie, 2015a), 0.38 mg/100 g for *V. amygdalina* (Usunobun and Okolie, 2015b), 0.75 mg/100 g for *S. macrocarpon* (Usunobun and Igwe, 2016a) and 0.85 mg/100 g for *C. argentea* (Usunobun

and Ekpemupolo, 2016), 0.97 mg/100 g for *C. odorata* (Usunobun and Ewere, 2016).

Manganese content of *A. hybridus* (1.21 mg/100 g) compared favorably with 0.83 mg/100 g for *A. muricata* (Usunobun and Okolie, 2015a), 0.81 mg/100 g for *C. odorata* (Usunobun and Ewere, 2016), 1.21 mg/100 g for *O. gratissimum* (Usunobun and Uwadiae, 2016) and 1.86 mg/100 g for *C. argentea* (Usunobun and Ekpemupolo, 2016) but high when compared to 0.4 mg/100 g of *P. mildbraedii* (Usunobun and Igwe, 2016b). According to Claude and Paule (1979), manganese is necessary for the functioning of the pituitary gland, the pineal gland, the brain, it promotes hepatorenal function, combats anaemia and also essential for growth.

The preliminary phytochemical tests performed on the *A. hybridus* leaves indicated the presence of saponins, tannins, flavonoids, and alkaloids. These bioactive constituents may be responsible for previously reported therapeutic effect of *A. hybridus*. Alkaloids and flavonoids act as powerful antioxidants which protect cells, prevent or repair damage done to red cells by free radicals or highly reactive oxygen species (Ogbe *et al.,* 2010). Flavonoids contain hydroxyls, responsible for radical scavenging effects of most plants. The mechanisms of action of flavonoids are through scavenging or chelating process (Pourmorad *et al*., 2006; Omale and Okafor 2008). Tannins possesses anti-helminthic (Molan *et al*., 2002), analgesic properties and also have anti-ulcer activities (Marhuenda *et al*., 1993).

Ascorbic acid also called Vitamin C is required for the prevention of scurvy and has many biological activities in the human body (Antonelli, 2002). Ascorbic acid contributes to antioxidant properties protecting erythrocyte membrane, maintaining the blood vessel flexibility and improving blood circulation in the arteries (Oboh, 2005). Ascorbic acid content of *A. hybridus* (154 mg/100 g) is high compared to 70 mg/100 g for *Amaranthus cruentus* (Oboh, 2005) but low compared to 400 mg/100 g for *Amaranthus caudatus* (Akindahunsi and Salawu, 2005).

DPPH radical scavenging activity has been extensively used for screening antioxidants ranging from fruits, cereals and vegetable juices or extracts (Ayoola *et al.,* 2006). DPPH is an unstable diamagnetic molecule that attains stability through protonation visually noticeable by an abrupt discoloration from purple to golden yellow. Our study on the radical scavenging activity of *A. hybridus* leaves using DPPH radical scavenging activity and reducing power showed that *A. hybridus* possess antioxidants which may be attributed to its phytochemical constituents such as flavonoids. In the reducing power activity, the presence of reductants in *A. hybridus* caused a reduction of $Fe^{3+}$ to the $Fe^{2+}$ form monitored by measuring the formation of Perl's Prussian blue colour at 700nm. Our findings using DPPH radical scavenging and reducing power are in agreement with previously published similar studies (Usunobun and Ekpemupolo, 2016; Usunobun and Igwe, 2016a, b; Usunobun and Uwadiae, 2016, Usunobun and Ewere, 2016).

In conclusion this study provides some experimental evidence for traditional use of *A. hybridus* in some painful conditions and in treating diverse

medical ailments. The use of *A. hybridus* in our community for soup making is also highly justified as the plant contains chemical constituents of pharmacological and nutritional significance. Efforts should also be made towards characterizing the entire bioactive agents' present in *A. hybridus* for full utilization.

## REFERENCES


Aiyegoro, O. A. and Okoh, A. I. (2010). Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary and Alternative Medicine* 10: 21 – 32.

Akindahunsi, A. A. and Salawu, S. O. 2005. Antioxidant indices of some green leafy vegetables. *Lebensmittel- Wissenschaft und Technologie* 38: 513–517.

Antonelli, M. L., D'Ascenzo, G., Lagna, A. and Pusceddu, P. (2002). Food Analysis: a new colorimetric method ascorbic acid (vitamin C) determination. *Talanta* 58: 29–36.

Ayoola, P. B. (2012). Trace Elements and Major Minerals of *Persea Americana, Mangifera indica*, and *Cocos Nucifera* Shell Charcoal. *World J of Engineering and Pure and Applied Sci*. 2(2): 81-86

Ayoola, G. A., Sofowora, T., Odukoya, O. and Coker, H. A. B. (2006). Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plant. *J. Pharm. Sci.* 8:133 –137.

Bhattacharjee, S. K. (2004). Handbook of Medicinal Plants, Pointer Publishers, Jaipur.

Claude, B. and Paule, S. (1979). The Manual of Natural Living. Edn 1, Biddles Ltd., Guildford, Surrey 98-101.

Cyrus, W. G., Daniel, G. W., Nanyingi, M. O., Njonge, F. K. and Mbaria, J. M. (2008). Antibacterial and cytotoxic activity of Kenyan medicinal plants. *Mem. Inst. Oswaldo Cruz* 103: 650.

Fakankun, O. A., Babayemi, J. O. and Utiaruk, J. J. (2013). Variations in the mineral composition and heavy metals content of *Moringa oleifera. African Journal of Environmental Science and Technology* 7(6): 372-379.

Gbolahan, D. (2001). Lesson Note on Medical Importance of Trace Elements. Centre for Natural Health Studies.

Krochmal, A., Walters, R. S. and Doughty, R. M. (1973). A Guide to Medicinal Plants of Appalachia, p.44n45, New York Times Book Co., New York.

Krochmal, A. and Krochmal, C. (1973). A Guide to Medicinal Plants of the United States, p.34, New York Times Book Co., New York.

Lichtenstein, A. H., Appel, L. J., Brands, M., Carnethon, M., Daniels, S., Franch, H. A., Franklin, B., Kris-Etherton, P., Harris, W. S., Howard, B., Karanja, N., Lefevre, M., Rudel, L., Sacks, F., Van Horn, L., Winston, M. and Wylie-Rosett, J. (2006). Summary of American heart association diet and lifestyle recommendations revision. *Arteriosclerosis, Thrombosis and Vascular Biology,* 26: 2186-2191.

Liyana-Pathiana, C. M. and Shahidi, F. (2005). Antioxidant activity of

commercial soft and hard wheat (*Triticum aestivium* L) as affected by gastric pH conditions. *Journal of Agricultural Food and Chemistry* 53: 2433 – 40.

Marhuenda, E., Marbin, M. J. and DelaCastra, C. A. (1993). Anti-ulcerogenic activity of Aescine in different experimental models. *Phytother. Res.,* **7**: 13.

Martin, F. W. and Telek, L. (1979). Vegetables for the hot humid. Part 6: Amaranth and Celosia. U.S. Dept of Agric., New Orleans, LA, pp.156-163.

Mepha, H. D., Eboh, L. and Banigbo, D. E. B. (2007). Effects of processing treatments on the Nutritive Composition and consumer acceptance of some Nigerian edible leafy vegetables. *Afr. J. Food Agric. Nutr. Dev.* 7(1): 1-18.

Molan, A. L., Waghorn, G. C. and McNabb, W. C. (2002). Effect of condensed tannins on egg hatching and larval development of *Trichostrongylus colubriformis in vitro*. *Vet. Rec*., 150: 65-69.

Nacoulma, O. G. (1996). *Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: cas du plateau central*; Thèse d'état, Université de Ouagadougou. p. 574.

Nielsen, S. S. (2010). *Vitamin C determination by Indophenol method and AOAC method*. Food Analysis Laboratory Manual, Food Science text series, Chapter 7, pp. 55–59.

Oboh, G. (2005). Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. *Lebensmittel-Wissenschaft und Technologie,* 38: 513–517.

O'Dell, B. L. (2000). Role of zinc in plasma membrane function. *Journal of Nutrition*, 130(5S): 1432S-1436S.

Ogbe, R. J., Adoga, G. I. and Abu, A. H. (2010). Antianemic potentials of some plant extracts on phenyl hydrazine-induced anaemia in rabbits. *Journal of Medicinal plants Research*, 4(8):680-684.

Oke, O. L. (1983). Amaranth. In: Handbook of Tropical Foods. Chan HT (ed). Marcel Dekker, Inc., New York, pp 1-14.

Oliveria, J. S., and DeCarvalho, M. F. (1975). Nutritional Value of some edible leaves in Mozambique. *Econ. Bot.* 29: 255-259.

Omale, J. and Okafor, P. N. (2008). Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata. Afr. J. Biotechnol.,* 7(17): 3129-3133.

Pourmorad, F., Hosseinimehr S. J. and Shanabi M. N. 2006. Antioxidant activity, Phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol.,* 5(11): 1142-1145.

Susan, A. and Lanham, N. (2008). The balance of bone health: tipping the scales in favour of potassium-rich, bicarbonate-rich foods. *J. Nutr.* 138: 172S-177S.

Trease, G. E. and Evans, W. C. (1985). *Pharmacognosy* 11th Ed., Tindall Ltd, London, pp60-75.

Usunobun, U., Okolie, N. P. Anyanwu, O. G., Adegbegi, A. J. J. and Egharevba, M. E. (2015).

Phytochemical screening and proximate composition of *Annona muricata* leaves. *European Journal of Botany, Plant Science and Phytology* 2: 18 – 28.

Usunobun, U. and Okolie, N. P. (2016). Phytochemical analysis and proximate composition of *Vernonia amygdalina. International Journal of Scientific World* 4: 11 – 14.

Usunobun, U. and Okolie, N. P. (2015a). Phytochemical analysis and mineral composition of *Annona muricata* leaves. *International Journal of Research and Current Development* 1:38 – 42.

Usunobun U. and Okolie N. P. 2015b. Phytochemical, trace and mineral composition of *Vernonia amygdalina* leaves. *International Journal of Biological and Pharmaceutical Research* 6: 393 – 399.

Usunobun, U. and Ekpemupolo, I. S. (2016). Phytochemical analysis, mineral composition and *in vitro* antioxidant activities of *Celosia argentea* leaves. *International Journal of Scientific World* 4: 19 – 22.

Usunobun, U. and Igwe, V. C. (2016a). Phytochemical analysis, mineral composition and *in vitro* antioxidant activities of *Solanum macrocarpon* leaves. *International Journal of Health*, 4(1): 62-65

Usunobun, U. and Igwe, V. C. (2016b). Phytochemical screening, mineral composition and *in vitro* antioxidant activities of *Pterocarpus mildbraedii* leaves. *International Journal of Scientific World* 4: 23 – 26.

Usunobun, U. and Uwadiae, E. (2016). *In vitro* medicinal studies on *Ocimum gratissimum* leaves. *ARC Journal of Pharmaceutical Sciences* 2(2):1-5.

Usunobun, U. and Ewere, G. E. (2016). Phytochemical analysis, Mineral Composition and *in vitro* antioxidant activities of *Chromolaena odorata* leaves. *ARC Journal of Pharmaceutical Sciences* 2(2): 6-10.