Determination of Phytochemical Composition, Mineral Content And Antioxidant Activity of Ethanol Extract of Amaranthus Caudatus

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Abstract- *This study was carried out to investigate the Determination of phytochemical Composition, Mineral Content and Antioxidant Activity of Ethanol Extract of Amaranthus caudatus. The level of phytochemical composition, mineral content and antioxidant activity were determined using standard analytical procedures. The quantitative phytochemical analysis of this plant showed that tannin was 3.99%, saponin 2.14%, alkaloid 2.18%, phenol 4.73mg/kg and flavonoid 2.84%. The extract also contained minerals, Iron 2.973ppm, Magnesium 23.18ppm, Sodium 49.728ppm, and Zinc 3.420ppm. The antioxidant activity of ABTS was 7.9602mg/ml, scavenging of nitric oxide 8.49mg/ml and lipid peroxide 0.35 x 10^{-5}mg/ml. These results show that its phytochemical composition justifies the ethnomedicinal importance of this plant and as well a good source of vital minerals and also enhances antioxidant activities which helps to mop up the free radicals in the biological system.*

Keywords- Antioxidant, Ethnomedicinal, Free radicals.

I. INTRODUCTION

Antioxidants are protective molecules, which trap and consequently protect the body from damage due to oxidative stress. Antioxidant neutralizes free radicals by accepting or donating an electron to eliminate the impaired condition. Thus they may well be defined as free radical stabilizers and quenchers. Antioxidant themselves do not become free radical by donating electrons because they are the antioxidant activities of natural substance which'-has been of interest in recent year. Antioxidant scavenges free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative disease (Cardado-Martinez, *et al.,* 2002). Ethanol, methanol and acetone are commonly used solvent to extract antioxidants from plant foods (Hazra, *et al.,* 2008). Bioactive components play a vital role in the treatment of various diseases; therefore it is important to identify, isolate, purify, characterized and quantify these bioactive components using various analytical method and also to determine their antioxidant and free radical scavenging capacity. Free radicals are chemical entities that can exist separate with one or more unpaired electrons produced from various biochemical reactions (Adedapo, *et al.,* 2009). They occur continuously in the cells as a result of enzymatic and non-enzymatic reactions to body. They are generated as a result of pro-oxidant in the body metabolic process (Hazra, *et al.,* 2008). Examples of these are reactive oxygen species (ROS) or reactive nitrogen species (RNS) radicals which include superoxide anions, singlet oxygen, hydrogen peroxide and hydroxyl radicals. These radicals can cause variety of pathological effects such as protein damage, DNA mutation, carcinogenesis, ageing, cardiovascular and neurodegerative diseases. They are implicated as a result of oxidative stress, occasional leakages from continuous exposure to chemicals, contaminants and exogenous factors (Gyanifi, *et al,* 2002). The pathological disruption of these radicals in the human cells could be prevented by quenching the upshort of catalytic activities. Synthetic drugs such as butylated hydroxytoluene (BHT), rutin, and butylated hydroxyl anisole (BHA) are commonly used. In view of the increasing risk factors, there has been a global trend toward the use of natural substance present in medicinal plants as therapeutic antioxidant agent (Gibson, *et al.,* 2006). Several studies have therefore demonstrated that plants produce potent phytochemicals with strong antioxidant activities and thus represent an important source of natural antioxidant (Gibson, *et al.,* 2006). Moreover, the use of natural products of plant origin has been proposed because they are natural antioxidants with rich sources of metabolites. They contain bioactive chemicals such as phenols, alkaloids and lignin which are potent radicals terminators that can help in reducing the risk of cancer toxicity, inflammation and cardiovascular diseases.

II. MATERIALS AND METHODS

Electric blender (Binatone),Weighing balance, Whatman's filter paper, Beakers,Measuring cylinders, Spatula, Spectrophotometer (Genesys 10-S. USA). Muffle furnance, Electric hot plate, Electric oven (Precision electrothermal model BNP.9052 England), 20% acetic acid, Methanol,

Ammonium hydroxide, Petroleum ether, Phenolphthaline, ABTS solution 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphoric acid), Phosphate buffer, Hydrogen peroxide, Sodium nitroprusside, Griess reagent,TBA (Thiobarbituric acid).The *Amaranthus* leaves were dried under the sun for 48 hours to reduce themoisture content. The leaves were grounded to powder using an electric blender and the sample were extracted with ethanol

Alkaloid determination

Five (5) grams of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hour at 25°c. This was filtered with filter paper NO.42 and the filtrate was concentrated using a water bath (Memmert) to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). Then, filter with pre-weighed filter paper. The residue on the filter paper is alkaloid, which is dried in the oven at 80°c. The alkaloid content was calculated and expressed as percentage of the weight of the sample analyzed

Calculation:

% weight of alkaloid = weight of filter paper with residue - weight of filter paper x 100 / weight of sample analyzed.

Flavonoid determination

Ten (10) grams of the leaf sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper NO.42 (125mm). The filtrate waslater transferred into a crucible and weighted into dry ness over a waterbath and weighed to a constant weight.

Calculation:

$$\% \text{ flavonoid} = \frac{(\text{weight of crucible + residue}) - (\text{weight of crucible}) \times 100}{\text{Weight of sample analyzed.}}$$

Saponin determination

Five (5) grams of the sample was put into 20% acetic acid in ethanol and allowed to stand in a waterbath at 50°c for 24hours. This was concentrated using a waterbath to one quarter of the original volume. Concentrated NH4OH was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The soponin content was weighed and calculated in percentage.

Calculation:

% saponin content = (weight of filter paper + residue) - (weight of filter paper) x **100** / weight of sample analyzed.

Tannin determination by titration

The follins dennis titrating method was used. 20g of crushed sample in a conical flask was added to **100**ml of petroleum ether and covered for 24hour. The sample was then filtered and allowed to stand for 15 min allowing petroleum ether to evaporate. It was the re-extracted by soakingin 100ml of 10% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected. 25ml of NH40H were added to the filtrate to precipitate the alkaloids. The alkaloid were heated with electric hot plate to remove some of the NH40H still in solution, the remaining volume was measured to be 33ml. 5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.1M NAOH using phenolphalyne as an indicator until a pink end point is reached. Tannin content was then calculated in % (C1V1=C2V2) molarity.

Calculation:

Data:

C1= concentration of tannic acid
C2= concentration of base
VI = volume of tannic acid
V2= volume of base

$$\text{Therefore } C1 = \frac{C2V2}{VI}$$

$$\% \text{ of tannic acid content} = \frac{Cl \times 100}{\text{Weight of sample analyzed}}$$

Phenol determination

The quantity of phenolic was determined using the spectrophotometer method. The leaf sample was boiled with 50ml of $(CH_3CH2)_20$ for 15 min. 5ml of boiled sample was then pipette into 50ml flask, and 10ml of distilledwater was added. After the addition of distilled water, 2ml of NH40H solution and 5ml of concentrated $CH_3(CH\mathbf{2})\mathbf{3}CH_2OH$ was added to the mixture. The sample was made up to the mark and left for 30min to reactfor colour development and measured at 505nm wavelength using spectrophotometer.

Dry preparation of sample for minerals

Five (5) grams of grounded leaf sample was collected and heated in a furnace for 2 hours at 550°C, then diluted with 20ml of 20% H_2S04, and filtered with paper and finally measured using F5240AA agilent atomic absorption spectroscopy.

Preparation of sample for antioxidant activity
ABTS scavenging effect

The antioxidant effect of the leaf was studied using ABTS radical cation decolourization assay method

Reagent:

ABTS solution (7nm with 2.45Mm ammonium persulphate)
Procedure:

ABTS radical cation (ABTS+) was produced by reacting ABTS solution (7Mm) with 2.45mM ammonium persulfate. The mixture was allowed to stand in the dark at room temperature for 12 hours before use. Aliquets(0.5ml) of the three different sample were added to 0.3ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm in a spectrophotometer and the percent inhibition was calculated using the formular.

Calculation:

% inhibition = (control -test x **100**
Control

Hydrogen peroxide scavenging effect

The ability of the leaf samples to scavenge hydrogen peroxide was assessed.

Reagent**:**

Phosphate buffer (0.1M, pH 7.4)
H_2SO_4 (40mM) in phosphate buffer.

A solution of H_20_2 (0.1M,pH7.4) was prepared in phosphate buffer. Leaf sample at the concentration of 10mg/10µl volume were added to H_20_2 solution (0.6ml) and the total volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nM in a spectrophotometer containing phosphate buffer without H_20_2 scavenging of the sample.
Calculation:

% scavenging of hydrogen peroxide = (Ao-Al) x 100
Ao

Ao = Absorbance of control
A1 = Absorbance in the presence of sample.

Nitric oxide scavenging activity. The extent of inhibition of nitric oxide radical generation in vitro followed.

Reagent:

Sodium nitoprussside (100Mm)
Phosphate buffer saline (p^H 7.4)
Grieess reagent (1% sulphanilamide, 2% H_3P0_4)
0.1% naphthylene diamine dihydrochloride

Calculation:

Scavenge of nitric oxide =Abs of sample x cone. Of std
Abs of std

Procedure:

The reaction was initiated by adding 2.0ml of sodium nitroprusside. 0.5ml of leaf sample (50mg) and incubated at 250C for 30min. griess reagent (0.5ml) was added and incubated for another 30 minute. Control tbube were prepared without the samples. The absorbance was read at 546nM against the reagent blank, in a spectrophotometer.

Assay of lipid peroxidation

The extent of lipid peroxidation was estimated

Reagent:

TCA (10%)
TBA (0.1M)
Phosphate buffer (0.12M, PH 7.2)

Calculation:

Abs xTotai reaction volume
Sample volume x Extinction

Procedure:

A 20% liver homogenate was prepared in phosphate buffer (PH 7.2). To 0.5ml of the homogenate, 1.0ml of TCA and 1.0ml of TBA were added and mixed thoroughly. The mixture was heated in a boiling waterbath for 20 minutes. The tube were centrifuged at 535nM in a spectrophotometer (Genes\ s $10^{'5}$. USA) against a blank containing all the reagent

except the homogenate. The MDA equivalents of the sample were calculated using the extinction coefficient 1.56 x 10^5 M-l cm

III. RESULTS AND DISCUSSION

Table 1: phytochemical composition of ethanol extract of *Amaranthus caudatus.*

Phytochemical	Yield
Alkaloid	2.18%
Saponin	2.14%
Flavonoid	2.84%
Tannin	3.99%
Phenol	4.73mg/kg

Table 4: Mineral elements composition of ethanol extract of Amaranthus caudatus

Elements	Concentration (PPM)
Iron	2.973
Magnesium	23.318
Sodium	49.728
Zinc	3.420

Table 5: Antioxidant activity of ethanol extract of *Amaranthus caudatus*

Antioxidant activity	Yield
ABTS	7.9602mg/ml
Scavenging of hydrogen peroxide	18.5520mg/ml
Scavenging of nitric oxide	8.4932mg/ml
Lipid peroxide	0.3525x 10^5mg/ml

Phytochemicals were detected quantitatively in an appreciable level in ethanol extract of *Amaranthus caudatus* alkaloid (2.18%), saponin (2.14%), flavonoid (2.84%), tannin (3.99%) and phenol (4.73mg/kg).This revealed that phytochemical are non-nutritive plant chemical that prevent the development of bacteria as in tannin and also used as anti-inflammatory agent which make them very useful for pharmaceutical industries. The following mineral were also detected, iron (92.973ppm) and the recommended daily requirement (RDA) of iron for man is 6-40mg/kg. Sodium content was (49.728ppm) which is used for acid-base balance in the body fluid. The zinc content was (3.420ppm) and the recommended daily for zinc is 13mg (Adedapo, *et al*, 2008). Magnesium content was (23.318ppm) and the RDA for Mg in human is 250-380mg/kg. This revealed that they are good source of minerals for nerve transmission, proper fluid balance and energy metabolism. Finally antioxidant were also detected; ABTS (7.9602mg/ml), scavenging of hydrogen peroxide (18.5520mg/ml), scavenging of nitric oxide (8.4932mg/ml) and lipid peroxide (0.3525 x 10"^{5} mg/ml). this shows that they can be used to balance the oxidative state of plant and animal which have beneficial health effects and also mop up free oxygen radical.

IV. CONCLUSION AND RECOMMENDATION

The result of this work showed that *Amaranthus caudatus* commonly consumed green leafy vegetable in Nigeria, contain bioactive components which are helpful in the prevention of some cardiovascular and age related diseases with substantial minerals for body building and some antioxidants that mop up free oxygen radicals.

The results of the analysis showed the extract from the leaf have some medicinal due to its phytochemical and mineral composition. Therefore the leaf may hereby recommended for futher research to determine other bioactive compounds inherent in the sample that confers on it its biological activities.

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