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GC-MS, Antimicrobial and *In vitro* Antioxidant Assay of the Leaf Extract of *Alternanthera dentata*

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Authors' contributions

This work was carried out in collaboration between both authors. Authors DA and RIU designed the study and wrote the protocol. Author DA performed the statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the phytochemical content, antimicrobial and antioxidant activity of the leaf extract of *Alternanthera dentata.*
Study Design: Ethanol was used to extract the plant material for GC-MS analysis and antimicrobial studies while methanol extract was used for the antioxidant study.
Place and Duration of Study: Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri and Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, between July 2013 and December 2013.
Methodology: Dried leaves of *Alternanthera dentata* were extracted in ethanol for 48 hours and concentrated under reduced pressure. Five microbial isolates were used to test the susceptibility of the extract. DPPH and FRAP assays were used to test the antioxidant capacity of the plant.
Results: Eleven phyto–constituents were identified from the leaves. The most abundant compound was hexadecanoic acid (31.60%), followed by phytol (24.61%), and octadecanoic acid (10.56%). Other compounds identified in the leaves were glycerin (5.83%), levoglucosan (2.49%), alpha-D-

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glucopyranoside (5.21%), tetradecanoic acid (1.27%), 1-octadecyne (2.47%), octadecanoic acid, ethyl ester (6.79%), 10-undeceyn-1-ol (6.67%) and hexadecanoic acid (2.51%). The extract showed low antioxidant activity compared to ciprofloxacin, used as standard while antioxidant activity of the extract was lower than that of ascorbic acid used as the standard. At a concentration of 400 mg/ml, the extract produced the optimum activity using FRAP assay.
Conclusion: This study has revealed that *A. dentata* is rich in many important phytochemicals which could be further isolated and characterized for drug production.

Keywords: Phytochemical; antioxidant activity; antimicrobial; ethnomedicine; compound; extract.

1. INTRODUCTION

Plants have remained the natural reservoir for food and medicines for centuries [1]. The growing interest in validation of some folklore claims as well as the need for development of new drugs with potency to combat resistant diseases has greatly influenced research into underutilized plants in our ecosystem. Bioactive components of plants are a guide for the development of new therapeutic regimens that will serve for the treatment and management of many infectious diseases bedevilling man at present [2]. This is responsible for the growing interest in medicinal plants research worldwide [3]. Also, majority of the over 250,000 genera of plants in the globe are yet to be evaluated for their bioactive components with a view to aid new drug formulation [4]. It is therefore imperative to research into underutilized plants for important bioactive ingredients for drug production. Plants bioactive components with antioxidant potentials have found useful application as additives in food and pharmaceutical industries in recent times [5]. Flavonoids and Phenolics are among the plants antioxidant compounds with anti-inflammatory, antihypertensive and anticancer properties [6].

Alternanthera dentata, is one of the plants used in traditional medicine that belongs to the family of *Amaranthaceae*. It is commonly known as purple knight, Joseph's coat, joy weed or calico weed. It is a tropical plant with dark purple foliage and a sprawling, vigorous growth habit. Aside its numerous unexploited medicinal uses, it is often used for ornamental purposes. In Ikwuano LGA, Abia state, the leaves are used to prepare porridges and believed to possess blood boosting properties. Abishek et al. [7] reported that it is rich in iron and protein. The bioactive constitutes of this plant have not been fully documented and this has necessitated this research.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh matured leaves of *Alternanthera dentata* were harvested from a natural population in Umuariaga and National Root Crops Research Institute Umudike, Ikwuano L.G.A, Abia state, Nigeria. Authentication of plant material was done by Mr Ibe Ndukwe, a forester in the Department of Forestry and Environmental Management, Micheal Okpara University of Agriculture, Umudike, Nigeria.

2.2 Sample Preparation

The leaves were cleaned, sorted and cut into smaller sizes with the aid of a kitchen knife. It was allowed to dry under shade. Thereafter, it was milled into fine powder with an electric, Thomas Willey milling machine. A portion of 50 g of the powdered leaves was weighed and soaked in ethanol for 48 hours and filtered. The filtrate was concentrated with a water bath at 40°C for 2 hours. Also, 40 g of the ground sample was soaked in methanol for 48 hours. Thereafter, it was filtered with whatmann No.1 filter paper and dried in a hot air oven at 40°C. The methanol extract was stored in a refrigerator at 4°C until time for antioxidant assay.

2.3 GC-MS Analysis

Gas chromatography analysis was performed using GC-MS SHIMADZU QP 2010, JAPAN gas chromatography 5890-11 with a fused GC column (OV- 101) coated with polymethyl silicon (0.25 nm x 50m) and the conditions were as follows: Temperature programming from 80-200°C held at 80°C for 1 minute, rate 5°C/ min and at 200°C for 20 mins. FID temperature 300°C, injection temperature 250°C, carrier gas nitrogen at a flow of 1 ml /min, split ratio 1:75. The column length was 30 cm with a diameter of 0.25 mm and the flow rate of 50 ml/min. the elutes were automatically passed into a mass

spectrometer with a dictator voltage set at 1.5 kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents were all analytical grade and procured from Merck, Germany.

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST). Then the phytochemicals were identified based on the hits returned after comparing the unknown peak value and chromatogram from GC-MS against the known chromatogram peak value from the NIST Library database. Subsequently, the details about their molecular formula, molecular weight, structure were also obtained [8-10].

2.4 Determination of the *In vitro* Antioxidant Activities of *A. dentata* Using 2, 2-diphenyl-1-picryllhydrazyl (DPPH) Photometric Assay

The free radical scavenging activity of the extract was analyzed by the DPPH Assay using spectrophotometer. A quantity of 2 ml of the extract at varying concentrations (50, 100, 200 and 400 µg/ml) were mixed with 0.5 mM DPPH (in 1 ml of methanol) in a cuvette. The mixture was kept in the dark for 30 minutes. Thereafter the absorbance was taken at 517 nm. Each concentration of the extract was prepared in triplicate and the percentage antioxidant activity was calculated as follows:

$$\% \text{ antioxidant activity} = 100 - [\{(\text{absorbance of sample} - \text{absorbance of blank}) \times 100\} \times (\text{absorbance of control})^{-1}]$$

A volume of 1 ml of methanol and 2.0 ml of the extract was used as the blank. Ascorbic acid (vitamin C) was used as reference standard [11].

2.5 Ferric Reducing Antioxidant Power (FRAP) Assay

The method described by Benzie and Strain [12] was employed. A volume of 3 ml of freshly prepared FRAP reagent and 100 µl of extract in methanol at concentrations of 25, 50, 100, 200 and 400 µg/ml was mixed. The reaction was monitored for 4 min at 593 nm using spectrophotometer (Spectrum labs, USA) at 37°C. The assay was done in triplicates. Ascorbic acid was used as standard. Calculations were made by using a standard curve.

FRAP value of sample (µMol) = (Changes in absorbance of sample from 4 mins- 0mins / Changes in absobance of standard from 4 min- 0 min) x (FRAP value of standard (1000 µm) / 1)

2.6 Antimicrobial Study

The test solution was prepared by dissolving 5g of the plant extract in 10ml of distilled water. Five species of bacteria were obtained from the stock culture of the Federal Medical Centre, Umuahia. The organisms include: *Staphylococus aureus, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis* and *Salmonella typhi*. The organisms were resuscitated in peptone waster, sub-cultured into nutrient agar medium and incubated at 37°C for 24 hours. Filter paper discs were placed in glass petri dishes and sterilized in hot air oven. The agar plates were inoculated with a standard inoculum of the test organisms. Then filter paper discs containing 0.5 ml plant extract was placed on the agar surface. The petri-dishes were then incubated at 37°C for 24 hours. After the incubation period, the diameter of the zones of inhibition were measured and recorded. Ciprofloxacin was used as the standard. The plates were incubated at 37°C for 24 hours to obtain zones of inhibition [13].

2.7 Statistical Analysis

Results were represented as Mean ± Standard Error of Mean. One way analysis of variance (ANOVA) was used to analyze the data.

3. RESULTS AND DISCUSSION

The ethanol extract of the leaves of *A. dentata* on GC/MS analysis yielded 11 peaks showing the presence of eleven compounds in the extract (Table 1). The presence of different phytochemicals in the leaf extract of this plant shows that the plant has much medicinal potential. Hexadecanoic acid, the most abundant compound in the extract has antioxidant properties [14]. Phytol, is a major acyclic diterpene alcohol and a precursor for vitamins E and K_1. It is an antimicrobial, anticancer, anti-inflammatory and diuretic agent [15]. Phytol being a component of chlorophyll is essential for plant biosynthesis [16]. I-octadecyne and 10-Undecyn-1-ol have antimicrobial properties. The presence of these compounds suggests that this

plant can be a good source of antioxidants. The antimicrobial activity of the extract was lower than the ciprofloxazin used as standard (Table 2).

Plants are good sources of antioxidants that serve as important nutraceuticals, preventing oxidative damage [17,18]. Phenolic compound present in most crude plant extracts is the basis for the antioxidant effects of most plants [19]. The antioxidants needed by the human body to complement the exogenously produced ones are generally gotten from plants such as vegetables, herbs and fruits [20]. DPPH antioxidant assay is based on the ability of an antioxidant to reduce DPPH radical to a yellow coloured compound, diphenylpicrylhydrazin [21]. This reaction depends on the hydrogen donating ability of the antioxidant. DPPH assay has been adjudged to be better than many other *in vitro* antioxidant assays in terms of stability, sensitivity, simplicity and feasibility [22]. From the result (Fig. 1), reduction of DPPH radical was evidenced by the disappearance of deep violet colour to light yellow as well as decrease in absorbance at 517 nm. The extract produced its optimum activity at a concentration of 400 mg/ml. The antioxidant activity was concentration dependent and lower than that of ascorbic acid that served as the control. Antimicrobial activity of the extract was low (Table 2).

The antioxidant activity of *A. dentata* was also evaluated using FRAP assay. It is based on reduction of TPTZeFe (III) complex to TPTZeFe (II) complex by an antioxidant [12]. Higher absorbance indicates higher ferric reducing power. The FRAP antioxidant assay showed increasing activity with increasing concentration of the extract (Table 3). The result showed that 400mg/ml concentration produced the optimum activity. FRAP assay is limited in that it only measures the reduction of Fe^{3+} to Fe^{2+} [12]. The overall antioxidant activity of this plant could be due to its inherent phytochemicals present as x-rayed by the GC-MS analysis.

Table 1. Phyto-components identified in the ethanol leaf extract of *A. dentata*

No	RT	Name of compound	Molecular formula	Molecular weight	Peak area %
1	6.338	Glycerin	$C_3H_8O_3$	92	5.83
2	14.479	Levoglucosan	$C_6H_{10}O_5$	162	2.49
3	16.069	Alpha-D-Glucopyranoside	$C_7H_{14}O_6$	194	5.21
4	16.918	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.27
5	17.435	1-Octadecyne	$C_{18}H_{34}$	250	2.47
6	20.561	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	6.79
7	20.874	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	31.60
8	22.754	Phytol	$C_{20}H_{40}O$	296	24.61
9	23.437	10-Undecyn-1-ol	$C_{11}H_{20}O$	168	6.67
10	23.651	Octadecanoic acid	$C_{20}H_{40}O_2$	312	10.56
11	27.498	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330	2.51

Table 2. Antimicrobial activity of ethanol extract of *A. dentata* leaf

Microbes tested	Zone of inhibition (mm)	Zone of inhibition for ciprofloxacin (Control)
Staphylococcus aureus	10	15.3
Klebsiella pneumoniae	Nil	11.3
Escherichia coli	Nil	12.5
Proteus mirabilis	7.0	14.0
Salmonella typhi	8.0	15.3

Results are means of duplicate determinations

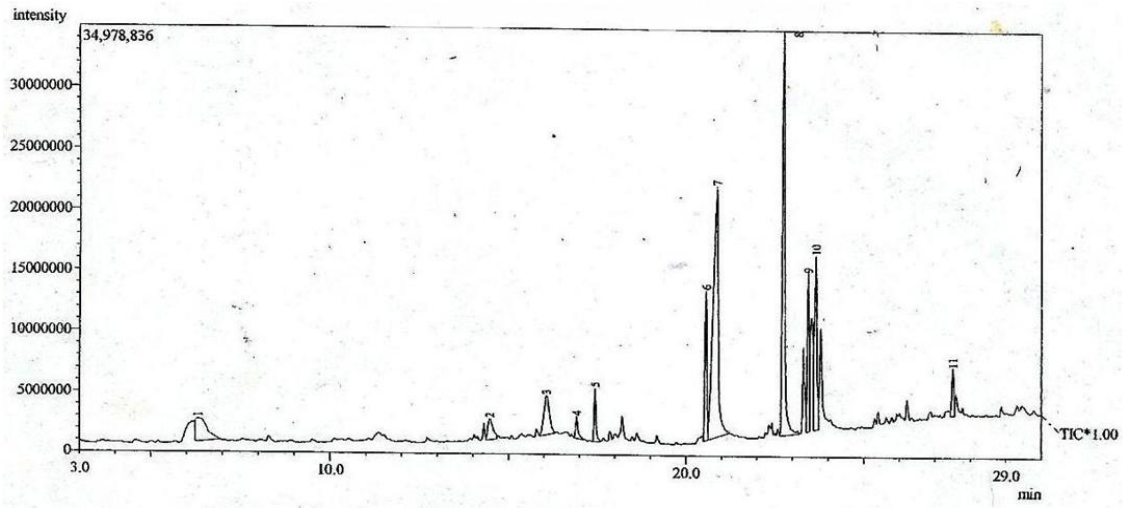

Fig. 1. GC-MS chromatogram of *A. dentata*

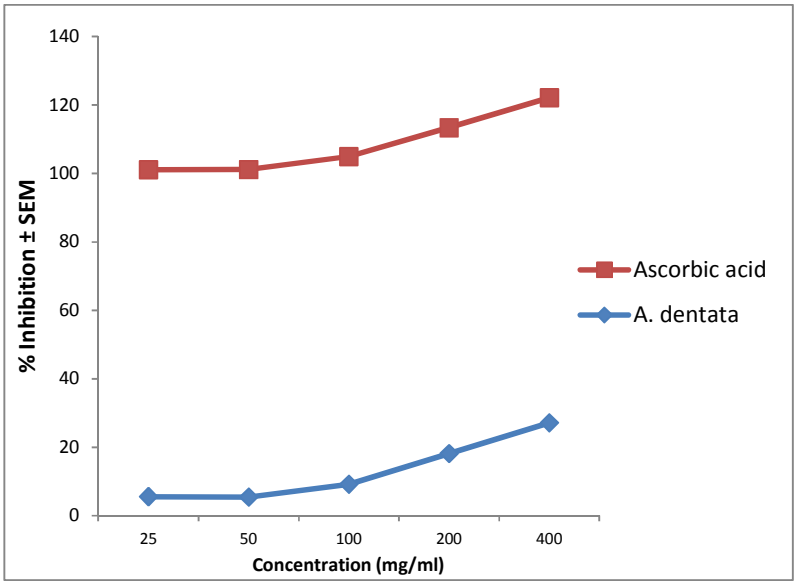

Fig. 2. Radical scavenging activity of *A. dentata* using DPPH

Table 3. Radical scavenging activity of *A. dentata* using FRAP

Concentration (µg/ml)	*A. dentata* (µmol/L)
25	0.65±0.01
50	1.60±0.09
100	4.67±0.22
200	9.09±0.24
400	16.20±0.26
Ascorbic acid (125 µg/ml)	2.00±0.00

**Values are represented as mean±SEM of triplicate determinations*

4. CONCLUSION

This study has revealed that *A. dentata* is rich in many important phytochemicals and antioxidant which could be further isolated and characterized for drug production.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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