

## Research Article

# Antioxidant Activity of *Acalypha wilkesiana* var. *lace-acalypha* (Muell & Arg.)

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**Background:** The incidence of free-radicals has become a major concern in health issues such as wounds, respiratory dysfunctions, heart troubles, cancers and inflammations amongst many others. The search for natural antioxidant drug templates led to the screening of *Acalypha wilkesiana* var. *lace-acalypha* for potential antioxidant activity.

**Objectives of study.** The crude extract, butanol fraction and four compounds previously obtained from the plant were to be screened for antioxidant activity.

**Methodology:** The extract, butanol fraction and four compounds designated as 5A-II (3), 5B (1), 5C (2) and 5D (H-2) obtained from silica-gel column chromatography of the butanol fraction were to be screened for antioxidant activity using DPPH reagent.

**Results and Discussion:** The extract and butanol fraction of *A. wilkesiana* var. *lace-acalypha* demonstrated marginal antioxidant activity (IC<sub>50</sub>) of at 0.79 and 0.77 µg mL<sup>-1</sup> respectively. However, 5B (1) and 5C (2) (both polyphenols) gave moderate antioxidant activities of 0.47 and 0.56 µg mL<sup>-1</sup> respectively which compare favourably with the IC<sub>50</sub> values obtained with vitamins C and A at 0.46 and 0.58 µg mL<sup>-1</sup> respectively. However, 5D (H-2) demonstrated a relatively similar antioxidant activity as vitamin E at 0.66 and 0.60 µg mL<sup>-1</sup> respectively. The antioxidant activities demonstrated by the plant extract/ fraction/compounds were not surprising because the phytochemical investigations carried out on the plant revealed the presence of terpenes and tannins which have been reported in previous studies to exhibit antioxidant activities.

**Conclusion:** The results of the antioxidant assays have revealed that compounds isolated from the plant especially 5B (1) and 5C (2) could serve as potential antioxidant templates which could be further investigated in detailed *in-vitro* and *in-vivo* studies with the aim of improving on their activities for the fights against the destructive free-radical oxygenated species.

**Keywords:** Free-radicals; antioxidant activity; DPPH; assay; *Acalypha wilkesiana*

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## 1. Introduction

Nature is blessed with enormous biodiversity resources of which plants form a huge component. Plants are used for food and remedy for ailments. Nutrition and health are interconnected and many plants are consumed as food in order to enhance health (Etkin, 1990; Pieroni, 2000; El and Karakaya, 2004; Ansari et al, 2005). Native plant species used in daily diets such as spices, condiments and additives are associated with a lowered incidence of degenerative diseases. These protective

effects are considered to be related to the antioxidants contained in them. The oxidative stress encountered by a cell, tissue, organelle or organ is a consequence of the balance between the production and removal of potentially damaging reactive oxygen species (ROS). Antioxidants are now associated with good health. They are a special class of compounds which prevent damage caused by excessive production of free-radicals, charged chemical species generated by various sources such as pesticides, smoking and exhaust fumes. The destruction of these radicals will assist in the fight against various

types of cancers, heart diseases, inflammations, radiation injuries and other immune-compromising diseases (Yi-Fang et al, 2002; Aruoma, 2003). Certain herbs and shrubs are now recognized as sources of natural antioxidants which can prevent oxidative stress and aid the chemosuppression of diseases that have their etiology and pathophysiology in reactive oxidative species (Lee and Shibamoto, 2002; Dragland et al, 2003; Odukoya et al, 2005a; Odukoya et al, 2005b; Odukoya et al, 2005c; Odukoya et al, 2007). One of such shrubs is *Acalypha wilkesiana* var. *lace-acalypha* (Muell & Arg.) which is used in the treatment of fever, skin fungal infections, wounds, tumors and inflammations (Oliver, 1959; Oliver, 1960; Sofowora, 2008).

This present study was carried out principally with the aim of prospecting for antioxidant activity in the extract, butanol fraction and isolated chemical compounds from the plant based on its reported uses in treating/managing tumors and inflammations.

## 2. Materials and Methods

### 2.1 Collection of Plant Material

The plant was identified and collected observing basic rules of plant collection. The fresh leaves of *A. wilkesiana* var. *lace-acalypha* were collected around the month of February, 2014 from a cultivated orchard at Nwaniba, Akwa Ibom State, Nigeria. The plant was identified at the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria. The authentication by comparison was done with herbarium samples of the Forestry Research Institute of Nigeria (FRIN) and the National Institute of Horticulture (NIHORT), Nigeria. A voucher specimen of the plant (**No H113**) was deposited in the Herbarium Unit of the Faculty of Pharmacy.

Immediately after collection, the plant was dried in an oven (Gallenkamp, England) at 40 °C for 48 h and the resultant dried material powdered on an electric mill (Uniscope, England).

### 2.2 Extraction and Processing of Plant Material

The principles governing extraction and processing of extracts were observed, thus preventing any changes to the chemical composition of the crude extract (Odebiyi and Sofowora, 1978; Odebiyi and Sofowora, 1979). The leaves were oven-dried (40 °C) and then ground into a coarse powder on an electric mill (Gallenkamp, England). The powder was subsequently extracted with cold 96 % ethanol at room temperature (27 ± 2 °C) for 72 h. The obtained filtrate was then evaporated to dryness *in-vacuo* on a rotary evaporator. A portion of the dried extract was dissolved in enough distilled water and repeatedly partitioned with butanol. The combined butanol fractions were bulked and likewise concentrated *in-vacuo* on a rotary evaporator.

The extract, resultant butanol fraction and isolates 5A-II(3), 5B (1), 5C (2) and 5D (H-2) previously obtained from the chromatographic separation of the butanol fraction were kept in appropriately labelled amber bottles and then stored in a refrigerator at -4 °C prior to the antioxidant tests.

## 2.3 Test for Antioxidant Activity

### 2.3.1 Initial Rapid Thin-layer Chromatographic DPPH (2,2-Diphenyl-1-picrylhydrazyl hydrate) Assay

This assay is premised on the principle of reduction. The purple color of the methanolic solution of DPPH is bleached when it accepts hydrogen or electrons from extracts or standard antioxidant drug. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) molecule is noted for its stable free radical nature and when mixed with a substance that can donate a hydrogen atom or electrons results in its reduced form, 1,1-Diphenyl-2-picrylhydrazine. The tests were done by developing the spotted samples of crude extract, butanol fraction (BU) and 5A-II(3), 5B (1), 5C (2) and 5D (H-2) in ethyl acetate: methanol (1:2) solvent mixture in duplicates. Ascorbic acid (Gemini Drugs, Nigeria) was spotted along to serve as positive control. The developed chromatograms were sprayed with 0.1 % w/v methanolic solution of DPPH reagent (Sigma-Aldrich, Germany). The plates were irradiated with ultra-violet light at  $\lambda_m$  366 nm for 15 minutes. Spots which appeared white against a purple background (Bondet et al, 1997; Cuendet et al, 1997; Kirby and Schmidt, 1997; Burits and Bucar, 2000) were taken as evidence of positive tests indicating anti-oxidant activity.

### 2.3.2 Spectrophotometric Determination of Antioxidant Activity Using DPPH Reagent

Substances capable of donating electrons or hydrogen atoms (free-radical scavengers) are able to convert the purple-colored DPPH radical (2, 2-Diphenyl-1-picrylhydrazyl hydrate) to its yellow-colored non-radical form (1, 1-Diphenyl-2-picryl hydrazine) (Guangrong et al, 2008; Nagalapur and Paramjyothi, 2010). This reaction can be monitored by spectrophotometry. This is the most widely employed method of screening for antioxidant activity in plants (Hu and Kitts, 2000; Khaled et al, 2002; Singh et al, 2002; Nia et al, 2003; Oladimeji et al, 2007; Guangrong et al, 2008; Nagalapur and Paramjyothi, 2010).

#### Preparation of Calibration Curve for DPPH Reagent

DPPH (4 mg) was weighed out and dissolved in methanol (100 mL) to produce the stock solution (0.004 % w/v). Serial dilutions were done to obtain the following concentrations; 0.0004, 0.0008, 0.0012, 0.0016, 0.0020, 0.0024, 0.0028, 0.0032 and 0.0036 % w/v. The absorbance of each of the sample was taken at  $\lambda_m$  512 nm (Bondet et al, 1997; Cuendet et al, 1997; Kirby and Smith, 1997; Burits and Bucar, 2000) using ultra-violet spectrophotometer (Jenway 6405, USA). This machine was zeroed after each absorbance measurement by a solution of methanol without DPPH which was used as the blank.

The absorbance of the DPPH solution increases as the concentration increases and obeys the Beer-Lambert law at concentrations of 50-100  $\mu$ M (Blois, 1958). Plotting of absorbance against concentration for a cell of unit thickness (1 cm) gives a straight line passing through the origin (Olaniyi, 1989; Olaniyi, 2000). In this way, the calibration curve for the DPPH reagent was prepared.

### Determination of the Antioxidant Activity of Crude Extract/Butanol Fraction/Isolates

Two mg of the extract/fraction/isolate from the plant was dissolved in 50 mL of methanol. Serial dilutions were done to obtain the following concentrations; 0.0008 mg mL<sup>-1</sup>, 0.0016 mg mL<sup>-1</sup> and 0.0024 mg mL<sup>-1</sup> using methanol. 5 mL of each concentration was incubated with 5 mL of 0.004 % w/v methanolic DPPH solution for optimal analytical accuracy (Bondet et al, 1997). After an incubation period of 30 minutes in the dark at room temperature (25 ± 2 °C), observation was made for a change in the color of the mixture from purple to yellow (Guangrong et al, 2008; Nagalapur and Paramjyothi, 2010). The absorbance of each of the test samples was then taken at λ<sub>m</sub> 512 nm (Bondet et al, 1997; Cuendet et al, 1997; Kirby and Smith, 1997; Burits and Bucar, 2000; Nia et al, 2003). The Radical Scavenging Activity (RSA %) or Percentage Inhibition (PI %) of free radical DPPH was thus calculated:

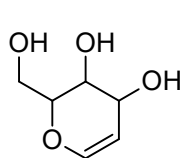
$$RSA \% (PI \%) = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

(Guangrong et al, 2008).

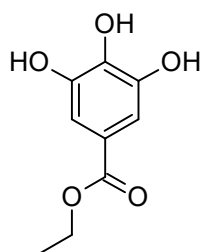
A<sub>blank</sub> is the absorbance of the control reaction (DPPH solution without the test sample);

A<sub>sample</sub> is the absorbance of DPPH incubated with the extract/fraction/isolate /anti-oxidant drug.

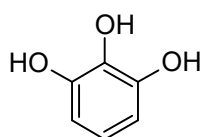
Extract/fraction/isolate /standard antioxidant drug concentration providing 50 % inhibition (IC<sub>50</sub>) was calculated using a graph of inhibition percentage against the concentration of the extract/fraction/isolate/standard antioxidant drug (Guangrong et al, 2008; Lebeau et al, 2000; Leitao et al, 2002).



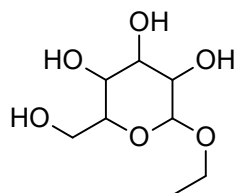
5A-II (3)



5B (1)



5C (2)



5D (H-2)

**Figure 1:** Chemical structures of compounds 5A-II (3), 5B (1), 5C (2) and 5D (H-2).

### DPPH Assay of Standard Antioxidant Drugs

Standard antioxidants namely, vitamin A (Orange Drugs, Nigeria), vitamin C (Greenfield Drugs, Nigeria) and vitamin E (Neimeth, Nigeria) were used. While vitamin C was in a tablet dosage form, vitamins A and E were formulated as gelatine capsules. The estimated weight of the formulations containing 2 mg of the standard antioxidant drugs were determined by proportionality and then diluted. Methanol was used to dissolve vitamin C, while n-hexane was used to dissolve vitamins A and E because solubility problems encountered with these two vitamins. Thus, methanolic and hexane solutions of 0.004 % w/v DPPH were used for incubation of vitamin C, vitamin A and E respectively for 30 minutes.

The absorbance value of each of the drugs was taken at wavelength at λ<sub>m</sub> 512 nm and the IC<sub>50</sub> determined.

### 3. Results

The butanol fraction (BU) obtained from solvent-partitioning of the aqueous solution of the crude extract was subjected to silica-gel column chromatography. This exercise led to the isolation of four compounds coded 5A-II (3), 5B (1), 5C (2) and 5D (H-2) (**Figure 1**). The identities of these compounds have been established to be D-arabino-hex-1-enitol-1, 5-anhydro-2-deoxy (1, 5-anhydro-2-deoxy-D-enopyranose-arabino-hex-1-enitol), ethyl 3, 4, 5-trihydroxybenzoate (ethyl gallate), 1, 2, 3-benzenetriol (pyrogallol) and ethyl α-D-glucopyranoside respectively using a combination of <sup>1</sup>H NMR, <sup>13</sup>C NMR, MS and IR spectral techniques (Oladimeji and Igboasoii, 2014; Oladimeji and Udom, 2014; Oladimeji and Johnson, 2015).

The extract/fraction/isolate and ascorbic acid gave white spots on purple background when the chromatogram was sprayed DPPH reagent. The observed white spots (irrespective of initial spotted color) are evidence of the reduction of the DPPH reagent (discoloration) by the by free-radical scavenger in the samples.

The reduction of the DPPH radical was determined by taking its absorption at a wavelength of λ<sub>m</sub> 512 nm. It was observed that the absorbance of DPPH decreased as the concentration of added free radical scavenger (extract/fraction/isolate/standard antioxidant drug) increased which suggested that the DPPH reagent was being reduced. The results of the reduction are as presented in **Table 1**. The Radical Scavenging Activity (RSA %) or Percentage Inhibition (PI %) and the IC<sub>50</sub> values of extract and standard antioxidant drugs were computed as **Table 2** shows. The RSA % is an indicator of the antioxidant activity of extract/fraction/isolate/standard antioxidant drug (Meir et al, 1995; Hu and Kitts, 2000; Khaled et al, 2002; Singh et al, 2002; Nia et al, 2003; Guangrong et al, 2008; Nagalapur and Paramjyothi, 2010). The determined IC<sub>50</sub> values for the crude extract and butanol fraction (BU) were marginal at 0.79 and 0.77 μg mL<sup>-1</sup> respectively as presented in **Table 2**. This shows that BU demonstrated a higher antioxidant activity than the extract. However, 5D (H-2) demonstrated a relatively similar antioxidant activity as vitamin E at 0.66 and 0.60 μg mL<sup>-1</sup> respectively.

**Table 1:** Absorbance of Samples Incubated with DPPH at Different Concentrations ( $\lambda_{\max}$  512 nm)

Sample	Absorbance		
	0.0008 mg mL <sup>-1</sup>	0.0016 mg mL <sup>-1</sup>	0.0024 mg mL <sup>-1</sup>
<i>A. wilkesiana</i> var. <i>lace-acalypha</i>	0.638	0.632	0.610
BU	0.324	0.320	0.275
5A-II (3)	0.352	0.347	0.331
5B (1)	0.115	0.092	0.072
5C (2)	0.158	0.154	0.152
5D (H-2)	0.269	0.258	0.245
Vitamin A	0.182	0.178	0.164
Vitamin C	0.105	0.082	0.064
Vitamin E	0.188	0.173	0.159

**Table 2:** Radical Scavenging Activity (Percentage Inhibition) of Samples at Different Concentrations and IC<sub>50</sub> of Samples

Sample	RSA % (PI %)			IC <sub>50</sub> (µg mL <sup>-1</sup> )
	0.0008 mg mL <sup>-1</sup>	0.0016 mg mL <sup>-1</sup>	0.0024 mg mL <sup>-1</sup>	
<i>A. wilkesiana</i> var. <i>lace-acalypha</i>	52.50	54.38	58.09	0.79
BU	52.69	53.27	59.83	0.77
5A-II (3)	48.57	49.29	51.64	NR
5B (1)	83.20	86.59	89.48	0.47
5C (2)	76.93	77.51	77.79	0.56
5D (H-2)	60.79	62.30	64.19	0.66
Vitamin A	73.39	73.98	76.02	0.58
Vitamin C	84.66	88.07	90.69	0.46
Vitamin E	72.53	74.76	76.57	0.60

Blank Absorbance of 0.004 % w/v Methanolic DPPH Reagent: 0.684

**Key:** for Table 2 and 3

BU = Butanol fraction;

5A-II (3) = *D*-arabino-hex-1-enitol-1, 5-anhydro-2-deoxy (1, 5-anhydro-2-deoxy-*D*-enopyranose-arabino-hex-1-enitol);

5B (1) = Ethyl 3,4,5-trihydroxybenzoate (ethyl gallate);

5C (2) = 1, 2, 3-benzenetriol (pyrogallol).

5D (H-2) = Ethyl  $\alpha$ -*D*-glucopyranoside;

RSA % (PI %) = Radical scavenging activity (Percentage Inhibition);

IC<sub>50</sub> = Concentration at which 50 % of DPPH is Scavenged or Inhibited;

NR = Not Regressed (Values could not be regressed from the % inhibition-concentration curves).

#### 4. Discussion

Previous studies on the crude extract of this plant revealed the presence of alkaloids, saponins, tannins, terpenes and cardiac glycosides while flavonoids, anthraquinones and cyanogenic glycosides were absent (Oladimeji, 1997; Oladimeji et al, 2012). Secondary metabolites such as saponins, cardiac glycosides, alkaloids and tannins have demonstrated in several previous studies (Hillar et al, 1990; Rios et al, Lamikanra et al, 1990; Burapadaja et al, 1995; Harouna et al, 1995; Aiyelaagbe et al, 1998; Adewunmi et al, 1998; Ibewuiké et al, 1998; Adesina et al, 2000) to be responsible for the cure or management of many ailments caused by microbes and different kinds of disease conditions in the ethno-medicine of plants.

BU demonstrated a higher antioxidant activity than the extract. This observation is not surprising because the butanol fraction was expected to be comparatively purer than the crude extract. 5B (1) and 5C (2) (both polyphenols) gave moderate antioxidant activities of 0.47 and 0.56  $\mu\text{g mL}^{-1}$  respectively which compare favourably with the  $\text{IC}_{50}$  values obtained with vitamins C and A at 0.46 and 0.58  $\mu\text{g mL}^{-1}$  respectively.

The antioxidant activities demonstrated by the plant extract/fraction/isolates were not surprising because different preparations of *A. wilkesiana* var. *laceacalypha* are used in ethnomedicine to treat/manage disease conditions such as wounds, inflammations and tumors amongst so many others (Dalziel, 1956; Oliver, 1959; Oliver, 1960; Watt and Breyer-Brandwijk, 1962; Sofowora, 2008; Evans, 2009). Also, the phytochemical screening of the crude extract of the plant indicated the presence of terpenes and tannins (Oladimeji, 1997; Oladimeji et al, 2012). Interestingly, these classes of compounds have been reported in previous studies to exhibit antioxidant activities (Tsimidou and Boskou, 1994; Lagouri and Boskou, 1995; Yokosawa et al, 1997; Daniel et al, 1998; Grassmann et al, 2001; Alemika et al, 2004; Malaya et al, 2004; Svoboda et al, 2006; Oladimeji et al, 2007). Furthermore, the importance of the radical scavenging ability of some phytochemical compounds have found useful applications in the extension of shelf-life and control of deterioration of fatty foods, nutraceuticals and spices (Thomas and Wade, 2001; Braca et al, 2003; Shahidi, 2000; Liyana-Pathirana and Shahidi, 2006).

#### 5. Conclusion

The results of this present study have revealed that compounds isolated from the plant have demonstrated antioxidant activities which compare favourably with those of standard antioxidant drugs. Also, these compounds could further be investigated in detailed *in-vitro* and *in-vivo* studies with a view to perfecting their antioxidant activities hence, serving as alternatives to the ones currently in clinical use on account of cheapness and with little or no toxicities.

#### Conflict of Interest Declaration

The authors declare no conflict of interest.

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