

Original Article

**IN VITRO ANTIOXIDANT AND FREE RADICAL SCAVENGING POTENTIAL OF METHANOLIC EXTRACTS OF *UVARIA CHAMAE* LEAVES AND ROOTS**

**NWOKOLO LORRETA NWAKAEGO<sup>a</sup>, ONYEKWELU KENECHUKWU CHIBUIKE<sup>a\*</sup>, ENE MARTIN CHUKWUGEKWU<sup>b</sup>, ADILIEJE CHIOMA MARYLYN<sup>a</sup>, EZECHUKWU IFUNANYA NGOZI<sup>a</sup>, EZEH RICHARD CHUKWUNONYE<sup>c</sup>**

<sup>a</sup>Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Enugu State Nigeria, <sup>b</sup>Department of Chemical Pathology, University of Nigeria Enugu Campus Medical Centre, Enugu State Nigeria, <sup>c</sup>Department of Medical Biochemistry, ESUT College of Medicine, Parklane, Enugu Nigeria  
Email: [kenechukwu.onyekwelu@unn.edu.ng](mailto:kenechukwu.onyekwelu@unn.edu.ng)

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ABSTRACT

**Objective:** This study investigated the *in vitro* antioxidant and free radical potentials of methanol extracts of *Uvaria chamae* leaves and roots.

**Methods:** Fresh *Uvaria chamae* leaves and roots were air dried, pulverized and extracted using methanol. Phytochemical, total phenolic, flavonoids, antioxidant and tannin contents, DPPH, hydroxyl, and superoxide radical scavenging properties of the extracts were determined using standard methods.

**Results:** *In vitro* antioxidant potentials revealed that methanol extract of *Uvaria chamae* leaves contains vitamin A (4871±79.21 I. U) and vitamin C (1.72±0.02%) while the root extract contains vitamin A (673.28±0.001. U) and vitamin C (1.66±0.01%). Both extracts had equal contents of vitamin E (8.83±0.04 mg/100g). The leaf extract scavenged 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) in a concentration dependent manner with the correlation coefficient (R<sup>2</sup>) of 0.839 and effective concentration (EC<sub>50</sub>) of 31.19 µg/ml, while the root extract scavenged DPPH with R<sup>2</sup> of 0.778 and EC<sub>50</sub> of 14.00 µg/ml. The leaf and root extracts scavenged superoxide radical and hydroxyl radical with EC<sub>50</sub> of 5.93 µg/ml and 719.45 µg/ml; 107.89 µg/ml and 912.01 µg/ml respectively compared to the EC<sub>50</sub> of ascorbic standard (30.27 µg/ml) and EC<sub>50</sub> of vitamin E standard (106.66µg/ml) respectively. The leaf extract showed significantly higher (*p*<0.05) anti radical power (ARP) of superoxide (0.17) compared to the root extract (0.0014) and the root extract showed significantly higher (*p*<0.05) ARP of DPPH (0.071) compared to the leaf extract (0.032).

**Conclusion:** The leaves and roots of *Uvaria chamae* are rich in natural antioxidants that can be exploited in the treatment of diseases related to oxidative stress.

**Keywords:** *Uvaria chamae*, DPPH, Superoxide radical, Hydroxyl radical

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INTRODUCTION

There are several types of antioxidants; natural antioxidants, which are obtained entirely from natural sources and the synthetic antioxidants which are created from chemical processes. Natural antioxidants from plant origin could provide alternatives to synthetic antioxidants. Antioxidants are nature's way of protecting the body and cells from damaging free radicals [1].

Reactive oxygen species (ROS) are highly reactive molecules possessing one or more unpaired electrons generated in living systems in the presence of molecular oxygen.

Mitochondria are the major site of free radical/ROS production in the body through oxidative phosphorylation resulting in the formation of ROS as a by-product of electron transfer reactions. Other endogenous sources of cellular ROS includes the phagocytic cells such as neutrophils, eosinophils and macrophages which generate ROS during the oxidative burst; the peroxisomes which produces hydrogen peroxide under physiological conditions and the cytochrome P450 enzymes which by oxidizing unsaturated fatty acids reduces molecular oxygen to superoxide anion radical and hydrogen peroxide [2-4].

The overproduction of ROS in biological systems leads to an imbalance between the formation of ROS and the antioxidant defence mechanisms, resulting in chemical modifications of cellular DNA, proteins or lipids, which lead to oxidative stress [5-7].

Oxidative stress is implicated in most human diseases. For instance, oxidatively damaged cell membranes lose their functional integrity that could lead to disease states while oxidatively damaged glucose and protein molecules form glycated proteins that can lead to cataracts and other diseases.

Natural antioxidants work by donating an electron to a molecule that has been compromised by oxidation, bringing it back into a state of proper function and having been used up in this way, the antioxidant molecule is either re-charged by accepting an electron from another type of antioxidant or it is re-cycled. Contrary to the natural antioxidants, the synthetic antioxidants cannot be recycled and re-used by the organism once they have donated their electron and as a result of this, they tend to turn into harmful metabolic by products that increase, rather than decreasing the total load of oxidative stress on the organism. Since efficient antioxidant protective mechanisms are crucial to inhibit oxidative stress, there has been an intensified search for natural antioxidant that could serve as a replacement for synthetic antioxidants which have some adverse effects in the body. As a result of this, we initiated this study to investigate the *in vitro* antioxidant and free radical potentials of methanol extracts of *Uvaria chamae* leaves and roots.

MATERIALS AND METHODS

Plant materials (*Uvaria chamae*)

The *Uvaria chamae* leaves and roots were sourced locally from Awlaw town in Oji River Local Government Area of Enugu State. These materials were identified and authenticated by Mr A. Ozioko of bioresources diversity and conservative programme (BDPC) unit, University of Nigeria. A specimen was deposited in the herbarium for future reference with a voucher number of UNH 471b.

Chemicals/Reagents

All chemicals used in this study were of analytical grade and products of Sigma Aldrich, (USA), British drug house (BDH) (England), Burgoyne (India), Harkin and Williams, (England),

Qualikems, (India), Fluka (Germany), May and Baker, (England), LOBA chemie, (India) and J. T Baker (USA).

### Extraction

Large quantity of *Uvaria chamae* leaves and roots were cut into small pieces and ground into fine powder using a dry grinder. The grounded samples were sieved to get uniform particle size, then kept in an air-tight container and stored for extraction. Methanol extraction was done according to the method of Mahanta and Murkherjee [8] with little modification. The extraction process was carried out by soaking about 200g of dried powder in 1000 ml of 80 % methanol for 24 h. Crude extract was obtained by first filtering through a muslin cloth and further clarified by filtration through filter paper (Whatman No.1). The extracts were concentrated using evaporator set at 50 °C to get rid of methanol completely. The extracts were kept in the refrigerator for further analysis.

### In vitro antioxidant assay

#### Phytochemical analysis

The dried ground leaves and roots were subjected to preliminary phytochemical analysis for flavonoids, tannins, carbohydrates/glycosides, saponins, resins and alkaloids using standard method as described by Ioan [9].

#### Determination of total phenolic contents

Total phenolics were determined using Folin-Ciocalteu reagent (FCR) as described by Velioglu *et al.* [10], with slight modifications. A 100 µl of the extracts dissolved in methanol (1 mg/ml) was mixed with 750 µl of Folin-Ciocalteu reagent (diluted 10-fold in dH<sub>2</sub>O) and allowed to stand at 22 °C for 5 min; 750 µl of Na<sub>2</sub>CO<sub>3</sub> (60 g/l) solution was then added to the mixture. After 90 min, the absorbance was measured at 725 nm. The phenolic content was evaluated from a gallic acid standard curve.

#### Determination of tannin contents

Tannin content in each sample was determined using insoluble polyvinyl-polyrrolidone (PVPP), which binds tannins as described by Makkar *et al.* [11]. A known volume, 1 ml of extract was dissolved in methanol (1 mg/ml), in which the total phenolics were determined, mixed with 100 mg PVPP, vortexed, left for 15 min at 4 °C and then centrifuged for 10 min at 3000 rpm. In the clear supernatant, the non-tannin phenolics were determined the same way as the total phenolics. Tannin content was calculated as a difference between total and non-tannin phenolic content.

#### Determination of flavonoids

The flavonoid content was determined according to the method described by Kumaran and Karunakaran [12] with slight modifications. A 100 µl of plant extracts in methanol (10 mg/ml) was mixed with 100 µl of 20% aluminium trichloride in methanol and a drop of acetic acid and then diluted with methanol to 5 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 100 µl of 20% aluminium trichloride in methanol and a drop of acetic acid and then diluted to 5 ml with methanol. The absorption of standard quercetin solution (0.5 mg/ml) in methanol was measured under the same conditions, and the amount of flavonoids in plant extracts in quercetin equivalents (QE) was calculated. Antioxidant vitamins A, C and E were determined using the method of Pearson [13] as described below:

#### Determination of vitamin A concentration

One gram (1 g) of the extract was weighed and macerated with 20 ml of petroleum ether. The filtrate was collected and evaporated to dryness followed by the addition of 0.2 ml of chloroform acetic anhydride. Two milliliter (2 ml) of trichloroacetic acid (TCA) was added. The absorbance was read at 620 nm and the amount of vitamin A (IU) calculated.

#### Determination of vitamin C concentration

One gram (1 g) of the extract was weighed and macerated with 10 ml of 0.4 g of oxalic acid and filtered. One milliliter (1 ml) of the filtrate was pipetted followed by the addition of 9 ml of

indolphenols' reagent. The absorbance was read at 520 nm, and the amount of vitamin C (%) in the extract was calculated.

#### Determination of vitamin E concentration

One gram (1 g) of the extract was weighed, macerated with 20 ml of ethanol and filtered. To 1 ml was added 2 ml of 0.2% ferric chloride in ethanol and 0.5 % α,α or 2,2 dipyrldyl and the volume made up to 5 ml with distilled water. The absorbance of the solution was then read at 520 nm and the amount of vitamin E (mg/100 g) was calculated.

#### DPPH radical scavenging assay

Scavenging activity of the extracts on DPPH free radicals was determined according to the method described by Gyamfi *et al.* [14] with slight modifications. A 2.0 ml solution of the leaf and root extracts at different concentrations diluted two-fold in methanol was mixed with 1.0 ml of 0.3 mmol DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. This was repeated for root extract. Blank solutions were prepared with each test sample solution (2.0 ml) and 1.0 ml of methanol while the negative control was 1.0 ml of 0.3 mmol DPPH solution plus 2.0 ml of methanol. L-ascorbic acid was used as positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with a UV-visible spectrophotometer. DPPH radical scavenging activity was calculated using the equation:

$$\% \text{ Inhibition} = 100 \% \times \left( \frac{A_0 - A_s}{A_0} \right)$$

Where  $A_0$  is the absorbance of the control, and  $A_s$  is the absorbance of the tested sample.

#### Hydroxyl radical (·OH) scavenging assay

Hydroxyl radical scavenging assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA was carried out as described by Elizabeth and Rao [15] with a slight modification. Hydroxyl radical was generated by the Fe<sup>3+</sup>-ascorbate-EDTA-H<sub>2</sub>O<sub>2</sub> system. The reaction mixture contained, in a final volume of 1 ml, 2-deoxy-2-ribose (2.8 mmol); KH<sub>2</sub>PO<sub>4</sub>-KOH buffer (20 mmol, pH 7.4); FeCl<sub>3</sub> (100 µM); EDTA (100 µM); H<sub>2</sub>O<sub>2</sub> (1.0 mmol); ascorbic acid (100 µM) and various concentrations of the test sample or reference compound. The mixture was incubated for an hour at 37 °C after which, 0.5 ml of the reaction mixture was added to 1 ml 2.8% TCA, then 1 ml 1% aqueous TBA was added, and the mixture was incubated again at 90 °C for 15 min to develop the color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. Vitamin C, a classical OH scavenger was used as a positive control. Percentage inhibition was evaluated by comparing the test and blank solutions.

#### Superoxide radical (O<sub>2</sub><sup>-</sup>) scavenging assay

Superoxide radical activity was measured by the reduction of nitro blue tetrazolium (NBT) according to the method described by Fontana [16]. The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce NBT to a purple formazan. The 1 ml reaction mixture contained phosphate buffer (20 mmol, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations of the sample solution. After incubation for 5 min at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated.

#### Scavenging properties of extracts on nitric oxide radical (NO)

Nitric oxide radical (NO·) generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci *et al.* [17]. Briefly, the reaction mixture (5.0 ml) containing SNP (5 mmol) in phosphate buffered saline (pH 7.3), with or without the plant extracts at different concentrations, were incubated at 25 °C for 180 min in front of a visible polychromatic light source (25 W tungsten lamp). The NO· radical thus generated interacted with oxygen to produce the nitrite ion (NO<sub>2</sub><sup>-</sup>) which was assayed at 30-minute intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1 % sulphanilamide in 5 % phosphoric acid and 0.1 % naphthylethylenediamine dihydrochloride). The absorbance of the chromophore (purple

azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was measured at 546 nm. The nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out at least three times and the data presented as an average of three independent determinations.

#### Statistical analysis

Data were reported as means±S. D, where appropriate. One-way analysis of variance (ANOVA) and correlation analysis was used to

analyze the experimental data. Differences were considered significant when  $p < 0.05$  and  $p < 0.01$ .

#### RESULTS

The qualitative phytochemical composition of *Uvaria chamae* leaves and roots showed that proteins, carbohydrates, and tannins were present in higher amount in the leaves while in roots, they were moderately present except tannin that was in trace amount. Saponin was present in higher amount in both the leaf and root. Flavonoids and steroid were present in trace amount in the leaf while in the root, flavonoids were not detected and steroids were moderately present (table 1).

**Table 1: Qualitative phytochemical composition of *Uvaria chamae* leaves and roots**

Phytochemicals	Bioavailability (Leaves)	Bioavailability (Root)
Protein	+++	++
Flavonoids	+	ND
Glycosides	++	++
Saponin	+++	+++
Alkaloid	++	+
Carbohydrates	+++	++
Tannins	+++	+
Steroids	+	++

Key: +Present in trace amount; ++Present in moderate amount; +++Present in high amount; NDNot detected

The result of quantitative analysis of *Uvaria chamae* showed that the leaf extract had higher contents of flavonoids and tannins when compared to

that of the root extract while the root extract showed higher contents of total phenolic content when compared to that of the leaf extract (table 2).

**Table 2: Quantitative phytochemical composition of methanol extracts of *Uvaria chamae***

	Leaves	Roots
Total Phenolic content	0.61±0.16 GAE	0.69±0.21 GAE
Tannins	0.44±0.15 GAE	0.32±0.20 GAE
Flavonoids	0.004±0.0001 mg QE	0.0013±0.0003 mgQE

Values are obtained as mean±SD. Each value is the mean of three (03) essays (n =3)

The antioxidant vitamins concentration of methanol extracts of *Uvaria chamae* leaves and roots showed that the leaf extract had the highest vitamin A concentration (4871±79.21 I. U) compared to the root extract (673.28±0.001. U) While that of the water-soluble

vitamin C concentration slightly varied with the values of 1.72±0.02% for the leaf extract and 1.66±0.01% for the root extract. Vitamin E concentrations were the same in both extracts with the values of 8.83±0.04 mg/100 g each.

**Table 3: Antioxidant vitamin contents of methanol extracts of *Uvaria chamae* leaves and roots**

Parameter	Values mean±standard deviation (SD)	
	Leaf extract	Root extract
Vitamin A	4871±79.21 IU	673.28±0.00 IU
Vitamin C	1.72±0.02%	1.66±0.01%
Vitamin E	8.83±0.04 mg/100g	8.83±0.04 mg/100g

Values are obtained as mean±SD. Each value is the mean of three (03) essays (n =3)

The scavenging effect of methanol extracts of *Uvaria chamae* leaves and roots on DPPH radical revealed that the leaf and root extracts scavenged DPPH radical in concentration-dependent manner with the highest percentage inhibition of 70.69±13.29 and 76.13±8.80 respectively at the concentration of 125 µg/ml. The effective concentration (EC<sub>50</sub>) at 50% inhibition is 31.19 µg/ml for the leaf

extract and 14.00 µg/ml for the root extract compared to that of vitamin C (ascorbic acid) standard with EC<sub>50</sub> of 25.29 µg/ml. Comparison of both leaf and root extracts against percentage inhibition showed a statistically significant positive correlation ( $P < 0.05$ ) with a correlation coefficient ( $R^2$ ) of 0.839 and 0.778 respectively (table 4).

**Table 4: Scavenging effect of methanol extracts (expressed in µg/ml) of *Uvaria chamae* leaves and roots on DPPH radical**

Concentration (µg/ml)	Percentage Inhibition (%) <sup>a</sup> mean±standard deviation (SD)	
	Leaf extract	Root extract vitamin C (standard)
125	70.69±13.29	76.13±8.80 96.83±0.02
62.5	62.39±13.82	68.76±13.35 95.32±0.14
31.25	51.28±9.54	65.30±13.33 67.48±5.14
15.625	41.28±8.64	55.79±6.77 34.86±0.34
7.8125	27.49±10.31	37.73±3.27 18.20±0.99
3.90625	9.69±3.34	23.75±3.04 9.02±1.79
1.95325	2.71±0.73	11.78±7.60 4.88±1.02

Values are obtained as mean±SD. Each value is the mean of three (03) essays (n =3), EC<sub>50</sub> for leaf extract =31.19 µg/ml, EC<sub>50</sub> for root extract=14.00 µg/ml, EC<sub>50</sub> for vitamin C (ascorbic acid) standard= 25.29 µg/ml.

The scavenging effects of methanol extracts of *Uvaria chamae* leaves and roots on superoxide radical revealed an EC<sub>50</sub> of 5.93 µg/ml and 719.45 µg/ml for the leaf and root extracts respectively compared to that of vitamin C (ascorbic acid) standard with an EC<sub>50</sub> of 30.27µg/ml. Comparison of the concentration of leaf extract against its percentage

inhibition showed a positive correlation that was statistically not significant (P>0.05) with a correlation coefficient (R<sup>2</sup>) of 0.139 while the comparison of the root extract concentration against its percentage inhibition showed a statistically negatively significant correlation (P<0.01) with a correlation coefficient of-0.855.

**Table 5: Scavenging effect of methanol extracts (expressed in µg/ml) of *Uvaria chamae* leaves and roots on superoxide radical**

Concentration (µg/ml)	Percentage Inhibition (%) <sup>a</sup> mean±standard deviation (SD)	
	Leaf extract	Root extract vitamin C (standard)
500	65.81±1.78	50.12±8.34 53.12±13.86
250	75.14±3.52	76.38±2.32 56.17±13.35
125	86.63±2.63	81.03±3.91 57.68±12.97
62.5	92.13±1.56	89.65±2.07 51.38±21.11
31.25	93.54±3.21	94.37±0.54 61.38±7.80
15.625	75.12±12.48	94.23±2.30 47.93±4.32
7.8125	52.04±22.36	88.16±5.18 44.69±8.55
3.90625	47.58±20.80	86.04±2.24 31.16±4.79
1.95325	34.43±12.72	73.18±4.65 17.61±7.03

Values are obtained as mean±SD. Each value is the mean of three (03) essays (n =3), EC<sub>50</sub> for leaf=5.93 µg/ml, EC<sub>50</sub> for root=719.45 µg/ml, EC<sub>50</sub> for vitamin C (ascorbic acid) standard= 30.27µg/ml.

The scavenging effects of methanol extracts on *Uvaria chamae* leaves and roots on hydroxyl radical showed a concentration-dependent inhibition with an effective concentration (EC<sub>50</sub>) of 107.89 µg/ml for the leaf extract and 912.01 µg/ml for the root extract compared to that of vitamin C (ascorbic acid) standard with EC<sub>50</sub> of 106.66µg/ml. The comparison of leaf extract concentration against the percentage

inhibition showed positive correlation that was statistically not significant (P>0.05) with correlation coefficient (R<sup>2</sup>) of 0.732 while the comparison of the root extract concentration against a percentage inhibition showed a positive correlation that was statistically significant (P<0.05) with a correlation coefficient (R<sup>2</sup>) of 0.738.

**Table 6: Scavenging effect of methanol extracts (expressed in µg/ml) of *Uvaria chamae* leaves and roots on hydroxyl radical**

Concentration (µg/ml)	Percentage Inhibition (%) <sup>a</sup> mean±standard deviation (S.D.)	
	Leaf extract	Root extract vitamin C (standard)
500	57.66±3.48	39.08±5.52 62.11±8.20
250	56.08±3.75	38.62±6.42 60.16±10.27
125	53.47±2.00	36.53±6.26 54.98±9.56
62.5	48.14±3.17	31.61±9.16 48.56±5.16
31.25	25.09±6.79	21.44±8.29 38.54±3.64
15.625	18.62±4.74	14.11±4.58 19.99±3.05
7.8125	12.34±3.38	10.50±3.02 12.98±1.57

Values are obtained as mean±SD. Each value is the mean of three (03) essays (n =3), EC<sub>50</sub> for leaf=107.89 µg/ml, EC<sub>50</sub> for root=912.01 µg/ml, EC<sub>50</sub> for vitamin C (ascorbic acid) standard= 106.66µg/ml.

The scavenging effects of 1000 µg/ml methanol extracts of *Uvaria chamae* leaves and roots on nitric oxide radical showed that at the 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup> and 150<sup>th</sup> min, there were significant reductions (p< 0.05) on nitric oxide radical showing scavenging effect of the leaf extract but no significant reduction at 120<sup>th</sup> and 180<sup>th</sup> min when compared to that of control. There were no significant reductions (p> 0.05) on nitric oxide radical by the root extract at 180<sup>th</sup> min while at another min, the root extract showed marked reductions on nitric oxide radical concentration compared to that of control. The vitamin E standard significantly reduced (<0.05) nitric oxide radical at 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup> and 150<sup>th</sup> min but no reduction at 120<sup>th</sup> and 180<sup>th</sup> min. However, the root extract (1000 µg/ml) scavenged nitric oxide radical more than the leaf extract and vitamin E standard at the same concentration when compared to that of control.

## DISCUSSION

Plants produce a significant amount of antioxidants such as phenolics, flavonoids, and polyphenolics to prevent the oxidative stress caused by reactive oxygen species. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG), tert-butyl hydroxyl quinone (TBHQ) protect against oxidative damage but they have some adverse side effects, and this has prompted mass campaign to search for natural foods, herbs, and spices that are rich in antioxidants. Many natural antioxidants have been isolated from

roots, leaves, stems, and barks of different plants [18-20]. The antioxidant and polyphenolic contents of *Uvaria chamae* seed [21], root bark [22] and root [23] have been determined whereas in this study, the antioxidant capacity and free radical scavenging potentials of *Uvaria chamae* leaves and roots were measured.

Higher concentration of Vitamin A and C with the values of 4871±79.21 I. U and 1.72±0.02% respectively in *Uvaria chamae* leaf extract could suggest its anti-radical scavenging activities more than the root extract with the values of 673.28±0.00I. U and 1.66±0.01% respectively. Several studies have reported that the scavenging property could be used in the management of free radical oriented diseases. However, the results suggest that *Uvaria chamae* leaf could be recommended as an antioxidant supplement and could be used to manage pro-oxidant-related ailments. Moreover, both plant parts contain an equal amount of vitamin E (8.83±0.04 mg/100g). Hence, the root extract could also serve in scavenging free radical since vitamin C works synergistically with vitamin E.

The result of the antioxidant potentials of the leaf extract showed rapid scavenging activity on the superoxide radical and hydroxyl radical while the root extract rapidly scavenged only DPPH and nitric oxide radicals. This could suggest that the leaf and root extracts contains some secondary antioxidant metabolites that could make both extracts medicinally relevant. It is reported by most

studies that the efficacies of antioxidants are often associated with their ability to scavenge stable, highly reactive, free radicals.

The concentration-dependent percentage inhibitions observed for both the leaf and root extracts on DPPH radical showed EC<sub>50</sub> of 31.19 µg/ml and 14.00 µg/ml respectively compared to the ascorbic acid standard with the EC<sub>50</sub> (25.29 µg/ml). Both the leaf and root extracts showed a statistically strong significant (p < 0.05) positive correlation, R<sup>2</sup> of 0.839 and 0.778 respectively suggesting moderate scavenging activities of both extracts against the DPPH radical.

The concentration-dependent percentage inhibitions observed for both the leaf and root extracts on superoxide radical showed EC<sub>50</sub> of 5.93 µg/ml and 719.45 µg/ml respectively compared to the ascorbic acid standard with the EC<sub>50</sub> of 30.27 µg/ml. Both the leaf and root extracts also showed no statistically significant (p > 0.05) positive correlation and a statistically significant (p ≤ 0.05) negative correlation respectively with correlation coefficient, R<sup>2</sup> of 0.139 and 0.855 respectively suggesting a moderate scavenging activity of the leaf extract more than the root extract against the superoxide radical.

The concentration-dependent percentage inhibitions observed for both the leaf and root extracts on hydroxyl radical showed an EC<sub>50</sub> of 107.89 µg/ml and 912.01 µg/ml respectively compared to the vitamin E standard with EC<sub>50</sub> of 106.66 µg/ml. Both the leaf and root extracts showed a positive correlation that is statistically not significant (p > 0.05) with a correlation coefficient (R<sup>2</sup>) of 0.732 and 0.738 suggesting moderate scavenging activities of the extracts against hydroxyl radical.

The scavenging activity of the extracts at the concentrations of 1000 µg/ml, 500 µg/ml and 250 µg/ml for nitric oxide radical could suggest that root extract is more effective than the leaf extract and the vitamin E standard at the concentrations of 1000 µg/ml and 250 µg/ml while at the concentration of 500 µg/ml, the leaf extract was more effective than the root extract and the vitamin E standard. All these results could suggest that both extracts could be useful in the management of free radical-related diseases.

In conclusion, the leaf extract of *Uvariae charmae* is rich in useful phytochemicals and possesses moderate antioxidant activity more than the root extract.

#### AUTHORS CONTRIBUTIONS

Nwokolo Lorreta Nwakaego, Onyekwelu Kenekwkw Chibuike and Ene Martin Chukwugekwu conceived and designed the experiments, performed laboratory analysis/experiments, data analysis and participated in the writing and editing of the manuscript.

Ezeh Richard Chukwunonye, Adilieje Chioma Marylyn and Ezechukwu Ifunanya Ngozi participated in the analysis of the data and preparation of the initial draft of the manuscript and helped in the laboratory experiments.

#### CONFLICTS OF INTERESTS

We have no conflict of interest to declare.

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