

Antioxidant Properties and Vitamin Profile of *Hyptis verticillata* Cultivated in Calabar, Cross River State, Nigeria

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ABSTRACT: The present study investigated the antioxidant properties as well the vitamin composition of the leaves of *Hyptis verticillata* cultivated in Calabar, Cross River State, Nigeria. The antioxidant activity of *H. verticillata* leaves indicated high catalase concentration (0.04±0.01M) and low levels of reduced glutathione, superoxide dismutase and malondialdehyde while the concentration of vitamins showed high concentration of vitamin C (2.63±0.12mg/100ml) with low concentrations of beta-carotene and vitamin E (at $3.0 \times 10^{-3} \pm 0.08$ mg/100ml and $0.05 \pm 8.1 \times 10^{-4}$ mg/100ml) respectively. We therefore, suggests that the consumption of leaves of *H. verticillata* in form of infusion could serve as a potential source of locally available antioxidants.

Keywords: Antioxidants, *Hyptis verticillata*, vitamins and infusion

1.0 Introduction

Free radicals are very unstable having unpaired electron; thus, are very reactive. They react with components of the cell and damage them, leading to several pathological conditions like cancer, cardiovascular disease (CVD), Alzheimer's and many other disease conditions (El-Missiry, 2012). Antioxidant elicits its effect by reducing the free radical's energy or by giving up some of its own electron for use by the free radical, thus making it stable. In this process the antioxidant gets oxidized in the place of the cellular macromolecules. They are present in many fruits, vegetables and grains and are categorized based on their chemical composition, for example phenolics, carotenoids, flavonoids and many others (Michaelides, 2015). The formation of reactive oxygen species (ROS) like superoxide, nitric oxide, hydrogen peroxide as by-products in the plant and human cells during their metabolic processes is highly inevitable (Pandey and Pandey-Rai, 2014). Therefore, cells that are under aerobic conditions are constantly exposed to ROS that are effectively handled by the antioxidant systems of the cell like superoxide dismutase, catalase, glutathione peroxidase and many exogenous free radical scavenging substances like vitamins E and C and the carotenoids without any adverse effect, thus

maintaining the ideal cellular and systemic health and well-being (FAO/WHO, 2001; El-Missiry, 2012).

However, anytime the balance between the production of ROS and the defense by antioxidants is lost, oxidative stress is eminent and this subsequently results to alterations in cellular functions, thus leading to different pathological conditions (El-Missiry, 2012). Recently there has been an increased interest globally to discover natural antioxidant with low or no side effects for use in preventive medicine and the food industry (Hossein *et al.*, 2015). Vitamins are important for the growth, and also uphold good health in the body. They are essential in the utilization of food macromolecules like carbohydrates, proteins and lipids by the body.

Hyptis verticillata Jacq. commonly called John Charles is a perennial plant that belongs to the family Lamiaceae also known as mint family. It is a medicinal plant that originates from Central America and has been used traditionally over a long period of time. It is fast spreading to other parts of America and the Caribbean countries (Picking *et al.*, 2013). The cultivation of this plant in Nigeria has not been documented in literature. The present study was designed to evaluate the antioxidant properties and some vitamin composition of the leaves of *H.*

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verticillata cultivated in Calabar, Cross River state, Nigeria.

2.0 Materials and Methods

2.1 Plant material and treatment

Hyptis verticillata was cultivated in a homestead garden in Calabar Municipality, Cross River State, Nigeria, from where the fresh leaves were harvested. The leaves were washed and air dried at room temperature for two weeks. The dried leaves were blended to fine powder and the powdered sample was subsequently extracted in varying reagents and used for analyses.

2.2 Chemicals and reagents

Ethanol, Diethyl ether, Thiobarbituric acid (TBA), Trichloroacetic acid (TCA) n- Hexane were purchased from Sigma-Aldrich (St. Louis, Mo, USA) while Ellman's reagent (DTNB) manufactured by Thermo Scientific (Meridian Road, Rockford, IL 61105 USA). The reagents and chemicals were all of analytical grade.

2.3 Determination of catalase (CAT)

The catalase activity was determined using the method of Beers and Sizer (Ogbunugafor *et al.*, 2012). Some (0.1ml) of the sample in phosphate buffer (50mM, pH 7.0) and 2.9ml 30mM H₂O₂ in phosphate buffer, pH 7.0, was put in the cuvette and absorbance was read at a wavelength of 240nm. The reaction mixture contained total volume of 3ml and the catalase activity was calculated using the extinction coefficient for H₂O₂ of 40.0M⁻¹cm⁻¹.

Concentration was calculated using the formula:

$$C = \frac{\Delta Abs \times \text{Total reaction mixture}}{EL \times \text{Volume of sample used}}$$

Where,

C= Concentration of activity

ΔAbs= Change in absorbance

E= Extinction coefficient

L= Light path

2.4 Determination of superoxide dismutase (SOD)

Superoxide dismutase activity was determined using the method of Sun and Sigma (Ogbunugafor *et al.*, 2012). Some (2.95ml) of the sodium carbonate buffer (0.05M, pH 10.2), 0.02ml of sample and 0.03ml of epinephrine in 0.005N HCl were used in the initiation of the reaction, then 2.95ml buffer, 0.03ml of epinephrine and 0.02ml of sample were put into the cuvette and the absorbance was read at 480nm. The reaction mixture contained a total volume of 3ml and the activity calculation was done using an extinction coefficient of 4020M⁻¹cm⁻¹.

Concentration of SOD activity was calculated using the formula:

$$C = \frac{\Delta Abs \times \text{Total reaction mixture}}{EL \times \text{Volume of sample used}}$$

Where,

C= Concentration of activity

ΔAbs= Change in absorbance

E= Extinction coefficient

L= Light path

2.5 Determination of malondialdehyde (MDA)

Malondialdehyde was determined colorimetrically using TBARS employing the method of Nichans and Samuelson (Latha and Pari, 2004). A quantity (2ml) of TBA-TCA-HCl reagent in a ratio of 1:1:1 (0.37% TBA, 0.25N HCl and 15% TCA) was used to treat 0.1ml of sample homogenate (Tris-HCl buffer, pH 7.5) and then was placed for 15 minutes in a water bath, subsequently cooled and then centrifuged at 3000rpm for 10 minutes. The absorbance of the clear supernatant was measured against a reference blank at a wavelength of 535nm.

Concentration of MDA activity was calculated using the formula:

$$C = \frac{\Delta Abs \times \text{Total reaction mixture}}{EL \times \text{Volume of sample used}}$$

Where,

C= Concentration of activity

ΔAbs= Change in absorbance

E= Extinction coefficient

L= Light path

2.6 Determination of reduced glutathione (GSH)

Reduced glutathione was determined using Ellman's method (Latha and Pari, 2004). Some (5ml) of 15percent TCA was added to 0.3g of sample, shaken in order to mix well and centrifuged at 3000rpm for 10minutes. Then, 0.5ml of Ellman reagent and 3.0ml of 0.2M sodium phosphate buffer, (pH 8.0) were used to treat 1.0ml of the supernatant for 10 minutes and put into a reference cuvette. The absorbance was read against blank at a wavelength of 412nm.

Concentration of GSH activity was calculated using the formula:

$$C = \frac{\text{Abs test} \times \text{conc. of std.}}{\text{Abs std.} \times 1}$$

Where,

Abs test= absorbance of test

Abs std.= absorbance of standard

Conc. of std.= concentration of standard

2.7 Determination of vitamin A (beta-carotene)

Vitamin A was determined spectrophotometrically using the method of AOAC (2006). Some (100ml) of diethyl ether was used in homogenizing 5g of the sample for 10 minutes and filtered. Then, 100ml of 95percent ethanol was added to the residue, shaken for 30 minutes and filtered. The two extracts were mixed together and was transferred into a separating funnel. Distilled water was added gradually from the walls of the funnel until separation occurred. The aqueous layer was run into a 250ml beaker and the yellowish layer was transferred into a volumetric

flask. The absorbance of the yellowish layer was measured against blank at 452nm wavelength in the spectrophotometer.

Vitamin A (beta-carotene) was calculated using the formula:

$$\text{Vitamin A (beta-carotene)} = \frac{T_1 - T_2 \times \text{Std conc} \times \text{DF}}{St_1 - St_2}$$

Where,

T₁= absorbance of blank (sample)

T₂= absorbance of sample

St₁= absorbance of blank (standard)

St₂= absorbance of standard (vitamin A)

Std conc= standard concentration

DF= dilution factor

2.8 Determination of vitamin C (ascorbic acid)

Ascorbic acid was determined by titration using the method described by Ene-Obong *et al.* (2016). A quantity (10g) of the sample was weighed into an extraction tube, mixed with 10ml of the extracting solution (metaphosphoric acid-acetic acid solution) and then shaken for 30 minutes in a mechanical shaker. The sample was filtered using a Whatman No. 42 filter paper into a 100ml volumetric flask and then made up to the mark with extractant. Then, 20ml of the extract was titrated with 0.02N CuSO₄ solution using 10ml of potassium iodide and starch solution as indicator. A test for the blank sample was also carried out using the extracting solution and the indicator. The value of the sample was then subtracted from the blank for the calculation.

Vitamin C was calculated using the formula:

$$\text{Vitamin C (ascorbic acid)} = \frac{L - B \times \text{Std conc.} \times \text{DF}}{Z - B}$$

Where,

L= standard vitamin C

B= blank

Z= sample

Std conc= standard concentration

DF= dilution factor

2.9 Determination of vitamin E (tocopherol)

Vitamin E was determined spectrophotometrically using the method described by Achikanu *et al.* (2013). Some (20ml) of n-hexane was used to macerate 1g of the original sample in a test tube for 10 minutes and then centrifuged for 10 minutes and subsequently filtered. Some (3ml) of the filtrate was then transferred into a dry test tube in duplicates and evaporated to dryness in a boiling water bath, after which 2ml of 0.5N alcoholic potassium hydroxide was added and boiled for 30 minutes in a water bath. Then, 3ml of n-hexane was added to the test tubes and shaken vigorously. The n-hexane was transferred into another set of test tubes and evaporated to dryness. A quantity (2ml) of ethanol was added to the residue and 1ml of 0.2percent ferric chloride in ethanol was also added. Then, 1ml of 0.5percent α¹ α¹-dipyridyl in ethanol was added to make it up to five milliliters. The solution was mixed and absorbance taken at 520nm against the blank.

Vitamin E was calculated using the formula:

$$\text{Vitamin E (tocopherol)} = \frac{\text{Abs sample} - \text{Abs blank} \times \text{Std conc}}$$

Abs standard

Where,

Abs sample = absorbance of sample

Abs blank = absorbance of blank

Std conc = standard concentration

Abs standard = absorbance of standard (vitamin E).

2.10 Statistical analysis

The data obtained from this study was presented as mean ± standard deviation of triplicates determinations.

3.0 Results

The results of antioxidant and vitamin concentrations in the leaves of *H.verticillata* are presented in tables (1- 2) below.

Table 1: Antioxidant activity of *H. verticillata* leaves cultivated in Calabar, Cross River State, Nigeria

Antioxidant	Activity (M)
Superoxide dismutase	$4.98 \times 10^{-5} \pm 2.16 \times 10^{-5}$
Catalase	0.04 ± 0.01
Malondialdehyde	$1.88 \times 10^{-5} \pm 3.51 \times 10^{-8}$
Reduced glutathione	$7.95 \times 10^{-4} \pm 5.13 \times 10^{-6}$

Results are expressed as mean ± standard deviation of triplicate determinations

H. verticillata leaves had very high catalase activity (0.04±0.01M) and low activities of reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) (at $7.95 \times 10^{-4} \pm 5.13 \times 10^{-6}$ M, $4.98 \times 10^{-5} \pm 2.16 \times 10^{-5}$ M and $1.88 \times 10^{-5} \pm 3.51 \times 10^{-8}$ M) respectively.

Table 2: Vitamin composition of *H. verticillata* leaves cultivated in Calabar, Cross River State, Nigeria

Vitamin	Concentration (mg/100ml)
Vitamin A (beta-carotene)	$3.0 \times 10^{-3} \pm 0.08$
Vitamin C (ascorbic acid)	2.63 ± 0.12
Vitamin E (tocopherol)	$0.05 \pm 8.1 \times 10^{-4}$

Results are expressed as mean \pm standard deviation of triplicate determinations

The vitamin levels in *H. verticillata* leaves indicated high concentration of vitamin C (at 2.63 ± 0.12 mg/100ml) with low concentrations of vitamins E and A (beta-carotene) with the values of $0.05 \pm 8.1 \times 10^{-4}$ mg/100ml and $3.0 \times 10^{-3} \pm 0.08$ mg/100ml respectively in *H. verticillata* leaves.

4.0 Discussion

Antioxidants are molecules that inhibit the oxidation of other molecules; they scavenge free radicals released during oxidation (El-Missiry, 2012) which are implicated in a variety of disease conditions including CVD and carcinogenesis to mention a few (Pandey and Pandey-Rai, 2014). There was an appreciable increase in the concentration of catalase activity while the activities of the other antioxidant enzymes were low in *H. Verticillata* leaves cultivated in Calabar.. Catalase usually catalyze the breakdown of hydrogen peroxide to water and oxygen with high turnover number because one molecule of catalase can degrade millions of hydrogen peroxide molecules per second (Goodsell, 2004). Our findings showed similar pattern to the report of Bahekar and Kale (2016) in the ethanolic leaf extracts of *Manihot esculenta* Crantz .The only exception is in the concentration of low superoxide dismutase and reduced glutathione. The malondialdehyde, which is a marker for oxidative stress, was found to have the lowest activity in *H. verticillata* leaves and this was similar to the work reported by Al-harbi (2016) on Naringenin effects on Arsenic-induced liver injury in rats. Malondialdehyde is among lipid peroxidation end products and increased production is linked to elevated free radicals which in turn result in deleterious conditions like formation of adducts when they react with DNA bases. These adducts can inhibit the translocation of RNA Polymerase II (Cline *et al.*, 2004; Gawet *et al.*, 2004; Farmer and Davoine, 2007).

Vitamin composition of the leaves of *H. verticillata* indicated high level of vitamin C when compared to the vitamins E and A. The result obtained in this study was in agreement with the work of Ajiboye *et al.* (2013) and Uraku and Nwankwo (2015) that reported high level of Vitamin C in leaves of *M. koenigii* and *Senecio bialfrae*, respectively. Vitamin C aids in intracellular substance formation throughout the body, including tooth dentine, collagen and bone matrix and it is also a powerful antioxidant that helps in the transportation and non-heme iron uptake at the mucosa, folic acid intermediate reduction as well as

cortisol synthesis (Padayatty *et al.*, 2003; Truswell, 2003; Vasudevan and Skreekumari, 2011; Acikanu *et al.*, 2013; Uraku *et al.*, 2015). Hence, its deficiency leads to blood capillaries fragility due to decreased amount of intracellular substance and manifests clinically as scurvy, hemorrhage of the mucous membrane of the mouth and gastrointestinal tract (Achikanu *et al.*, 2013; Uraku *et al.*, 2015). Vitamin E level in some green leafy vegetables grown in southern Nigeria as reported by Achikanu *et al.* (2013), Ajiboye *et al.* (2013) and Uraku *et al.* (2015) indicated higher concentrations compared to *H. verticillata*. Vitamin E is a potent antioxidant that plays a role in the protection of cells from free radical-induced damage and it is also crucial in the normal function and formation of muscles and red blood cell (Lukaski, 2004). Vitamin A is very essential in the maintenance of the epithelial cell function, hence its importance in normal vision, gene expression, growth and immune function (Lukaski, 2004).

5.0 Conclusion

From the study, it can be concluded that *H. verticillata* leaves cultivated in Calabar, Cross River State, Nigeria show probable antioxidant activity that could be exploited as alternative source of local supplements in nutrition.

Conflict of interests

The authors did not declare any conflict of interest.

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