GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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# Phytochemical composition, *in vitro* antioxidant and alpha amylase inhibitory properties of Celosia leptostachya

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GSC Biological and Pharmaceutical Sciences, 2023, 22(02), 030–039

Publication history: Received on 26 October 2022; revised on 08 February 2023; accepted on 11 February 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.22.2.0411

**Abstract**

Plant based phytoconstituents of great importance in the development of drugs and treatment of several health diseases.In this study, the phytochemical constituents of ethanol extract of *Celosia leptostachya* leaves, *invitro* antioxidant and alpha amylase inhibitory properties were investigated. Gas chromatography-mass spectrum analysis was used to identify varying percentage composition of phytochemical constituents in the extract. *In vitro* models were used to evaluate the ability of varying concentrations of the ethanol extracts to exhibit antioxidant activity by free radical scavenging using nitric oxide (NO) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Major constituents are cis-3-Butyl-4-vinyl-cyclo pentene n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z) , Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene, octadecanoic acid (7.47%), arachidonic acid and 9-octadecenoic acid (E) (5.40%). Many of the constituents are also representative compounds belonging to fatty acid esters and volatile oils. *Invitro* antioxidant studies using DPPH and NO radical scavenging activity indicated that the extract exhibited > 50% inhibition at concentrations of 500 µg/ml and 600 µg/ml. IC50 values obtained for the extracts indicated strong antioxidant activity. Alpha amylase inhibition showed that increase in concentration of the extract exhibited a corresponding increase in percentage inhibition of the enzyme similar to acarbose used as standard. The kinetic studies under inhibitory conditions showed a decrease in Km and Vmax from slope and noncompetitive. Thus, it can be deduced that ethanol extract of *Celosia leptostachya* leaves contained essential phytochemical components that can be explored for broad spectrum bioactivity, pharmacological and nutritional applications. The inhibition mechanism is noncompetitive and can be utilized as strategy in control and treatment of hyperglycemia.

**Keywords:** Phytochemicals; Alpha amylase; Antioxidant; Inhibition; *Celosia leptostachya* leaves

## 1. Introduction

Antioxidants are substances that are known to reduce the damaging effects of reactive oxygen species (ROS) [1]. During oxidative stress, ROS are generated and increase the risk of developing many negative health diseases such as cardiovascular diseases, certain type of cancer, hyperglycemia, diabetes and rapid aging [2].

Plants have been identified as major source of therapeutic antioxidants due to the presence of essential phytochemical components contained in them [3]. Several studies have been conducted to evaluate the antioxidant potential of a wide variety of plants and its products as free radicalquenchers and scavengers, hydrogen and electron donors, peroxides and singlet oxygen decomposers, metal chelators and enzyme inhibitors [4, 5, 6, 7, 8]. Due to increased risk of humans

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to develop degenerative diseases over time continuous replenishing of therapeutic antioxidants becomes essential to improve quality of life [9, 10].

The plant, *Celosia leptostachya (C. leptostachya)* belongs to the Amaranthacea family. It is an annual weak erect herb widely distributed in Sub- Saharan Africa and grows very well during the wet season to a height of about 12 cm [11]. In Nigeria it is one of the most widely used herb for the management of many illnesses such as diarrhea, blood disease, tape worm, boils and eye infections [12]. It is commonly called 'Nnanafaa', 'sokoyokota' in Hausa and 'ajefowo' in Yoruba [13, 14].

In recent times, there has been growing interest in oxidative stress and implication in specific diseases which can be prevented by plant derived antioxidants, the study was therefore conducted to investigate the phytochemical constituents present in *Celosia leptostachya*, antioxidant and alpha amylase inhibitory properties.

## 2. Material and methods

### 2.1. Collection and Identification of Plant Materials

The leaves of *Celosia leptostachya* were randomly obtained from Choba Campus, University of Port Harcourt, Nigeria. They were identified at the Plant Science and Biotechnology Department, Rivers State University. The leaves were thoroughly cleaned and air dried for two (2) weeks. The dried leaves were then ground into a fine powder.

### 2.2. Chemical and Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl radical), Hydrogen Peroxide, Sodium Nitroprusside, Alpha amylase, 3, 5-dinitrosalicylic acid (DNS) and Acarbose were obtained from Inqaba BIOtec, West African LTD. Other chemicals and reagents used in the study were of analytical grade from Merck Chemicals (Darmstadt, Germany) obtained from the Department of Biochemistry, Rivers State University, Nigeria.

### 2.3. Preparation of Ethanol Extract of *Celosia. Leptostachya* Leaves

The powdered plant material (200 g) was macerated in 1000 ml of ethanol for 72 hours with intermittent shaking. The mixtures were filtered using what man No. 1 filter paper and concentrated to dryness at room temperature. The prepared extract was weighed and reconstituted in dimethyl sulfoxide to give a suitable stock solution of concentration (10mg/ml) for the study.

### 2.4. GC-MS Analysis of the Ethanol Extract of *Celosia leptostachya* Leaves

The powdered plant material was extracted in dichloromethane after soaking for a period of five days. 10 g of the sample was weighed and transferred into a beaker and 20 ml of the organic solvent was added. The mixtures were vigorously agitated and allowed to stand for another five days. The mixture was filtered and the process repeatedly carried out for two more consecutive times. The mixtures were combined and then collected and concentrated on a steam bath. 5.0 ml of the extracted mixture was collected and purified by passing through a pasture pipette packed with silica gel and anhydrous sodium sulphate and air dried for phytochemical investigation which was performed on a gas chromatographic Model: 7890A (GC) interfaced with Mass Selective Detector model: 5975C (MSD). The electron ionization energy was set at 70v with ion source temperature at 250 ℃. Pure helium gas (99.9% purity) was used as carrier gas, while HP-5 (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature was operated at 60 ℃ held for 0.5 minutes and ramped to 140 ℃ at the rate of 4 ℃/minutes, then ramped to 280 ℃ while holding for 5 minutes at the rate of 8 ℃ /minutes. The injection volume was 1.0 µl. The individual compounds were identified using the Chem-Office software attached to the MS library. The names and molecular formula of the components were ascertained using the database of National Institute of Standard and Technology (NIST).

### 2.5. Nitric oxide Scavenging Activity

The NO scavenging activity was evaluated according to the method described by [15] with slight modification. Sodium nitroprusside in buffer solution of pH 7.4 spontaneously generates nitric oxide which interacts with oxygen to produce nitric ions that can be measured using Griess reagent. 4.0 ml of Sodium nitroprusside (10 mmol/l) and 1ml of phosphate buffer saline pH 7.4 were mixed with 1ml of the different concentrations of the extract (0, 100, 200, 300, 400 500, 600) µg/ml and incubated at 25 °C for 150 minutes. 1.5 ml of the incubated mixture was added to 1.5 ml of Griess reagent (1% sulphanilamide, 0.1% napthylethylenediaminedihydrochloride (NED) and 2% $H_3PO_{4)}$ and left for 30 minutes at 25°C. The absorbance of the chromophoricazo-derivative formed was read at 546 nm. The absorbance of standard

solutions of ascorbic acid treated in the same way with Griess reagent was used as control. The experiments were performed in triplicates, and scavenging activity was expressed as percentage radical scavenging activity using equation (1). Percentage inhibition and regression curves were plotted and the linear equation obtained was applied for the determination of IC50 value**.**

$$\% \text{ NO Radical Scavenging Activity} = (Ac - As)//Ac \times 100 \quad \ldots\ldots\ldots (1)$$

Where, Ac is the absorbance of the control, As is the absorbance of the extract

### 2.6. DPPH Scavenging Activity

The scavenging ability of n-hexane and ethanol extract of the plant on DPPH radical was estimated in Solution of DPPH using the method described by [1]. (0.1mM) in methanol was prepared by dissolving 4 mg of DPPH in methanol and volume was made up to 100 ml with methanol. 3.0 ml of DPPH solution was added to 1.0 ml of different concentrations of extracts (0, 100, 200, 300, 400 and 600) µg/ml. The solution was kept in darkness for 30 minutes to complete the reaction. The mixture was measured spectrophotometrically at 517 nm. A standard of ascorbic acid was used as a positive control using same concentrations as that of extracts. The experiments were performed in triplicates, and scavenging activity was expressed as percentage radical scavenging activity using equation (2). Percentage inhibition and regression curves were plotted and the linear equation obtained was applied for the determination of IC50 value**.**

$$\% \text{ DPPH Radical Scavenging Activity} = (Ac - As)//Ac \times 100 \quad \ldots\ldots\ldots (2)$$

Where, Ac is the absorbance of the control, As is the absorbance of the extract.

### 2.7. Alpha-amylase Inhibitory Activity

The activity of amylase was assayed by the method described by [16] with slight modification. 400 µl of varying concentrations of the plant extract (100, 200, 300, 400, 500, 600) µg/ml. 400 µl of sodium phosphate buffer (50mM, pH 7.3) and 200 µl of the enzyme (5mg of α- amylase in 10ml of 50mM sodium phosphate buffer) were incubated at 37 ℃ for 10 minutes. After the incubation, 1.0 ml of 1% starch solution in buffer solution was added to each test tube and incubated for 10 minutes. The reaction was stopped with 1.0 ml of DNSA reagent (1g 3,5- dinitrosalicyclic acid, 30g sodium potassium tartrate and 20µl of sodium hydroxide to a final volume of 100 ml in distilled water). Test tubes kept in a boiling water bath for 10 minutes. The mixtures were allowed to cool at room temperature. The reaction mixtures were further diluted with 2.0 ml distilled water and the absorbance was recorded at 540 nm. Mean values were obtained from triplicate experiments. The control samples were prepared without plant extract. From the absorbance obtained, percentage inhibition and regression curves were made, and the linear equation was applied for the determination of IC50 value**.** The inhibition % was calculated by using equation (3).

$$\text{Inhibition } \% = \frac{AC - AS\ X\ 100}{AC} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

### 2.8. Preparation of Standard

Ascorbic acid and Acarbose were used as standard for the antioxidant and alpha amylase inhibitory assay. A stock solution with a concentration of 10 mg/ml were prepared in each case and was made into several final concentrations (100, 200, 300, 400, 500, 600) µg/ml.

### 2.9. Alpha- amylase Inhibition Kinetics

The mechanism of inhibition of alpha-amylase was conducted according to the method of [17] with slight modifications. The inhibition type was determined using Lineweaver -Burk equation and double reciprocal plots. Initial velocities ($V_0$) were determined with starch at varying concentrations (100,300,500 and 700) µg/ml with or without plant extract. 400µL of plant extract at IC50 (5.88mg/ml) was incubated with 400µL of α-amylase solution at 37 ℃ for 10 min. In another set of assays, 400µL of phosphate buffer (50mM, pH 7.3) was incubated with 400 µL of α-amylase. Then, 200 µl of increasing concentrations of starch solution were added to the both sets of test tubes to start the reaction and then kept at room temperature for 10 minutes. 500 µl of DNS was added to the reaction and then boiled for 10 minutes. Double reciprocal (1/Vo) versus 1/[So] of Lineweaver–Burk were plotted used to determine the enzyme kinetic parameters (Km, Vm and Ki) and kinetic inhibition pattern.

### 2.10. Statistical Analysis of Data

The data obtained from this study were statistically analyzed by SPSS software version 2.0. One-way ANOVA was applied for evaluating the significant differences followed by Kruskal-Wallis H test for the establishment of significant difference.

## 3. Results and Discussion

Plants contain wide range of chemical components. The amount of the chemical components differs from one plant to another and also in different parts of the plant [18]. The plant derived chemical compounds are called Phytochemicals. In this study, the phytochemical constituents determined showed variation in composition in the ethanol extracts of *Celosia leptostachya* as shown in Table 1. Major constituents are cis-3-Butyl-4-vinyl-cyclo pentene (18.26 %), n-Hexadecanoic acid (17.97%), 9,12-Octadecadienoicacid (Z,Z)(12.4%),Bicyclo [2.2.1]heptane7,7-dimethyl-2-methylene(9.05%), Octadecanoic acid (7.47%), Arachidonic acid (5.84%), 9-octadecenoic acid (E) (5.40%). Constituents are also representative compounds belonging to fatty acid esters and volatile oils like octadecenoic acid, eicosanoic acid, octadecanoic acid, hexadecenoic acid pentyl ester. Other minor constituents include trans-4-amino-cyclohexanol, 2,4-Nonadienal, (E,E), diethylphthalate, N-butyl- Benzenesulfonamide,4,5-Octanediol, 1,1,1,5,7,7,7-Heptamethyl 3,3 bis (trimethylsiloxy) tetrasiloxane.The phytochemical constituents found in the extract can be explored for broad spectrum bioactivity, pharmacological and nutritional applications [19, 20]. Many chiral amino alcohols serve as building blocks for the development of drugs against cardiovascular, inflammatory, renal and hepatic diseases [21].

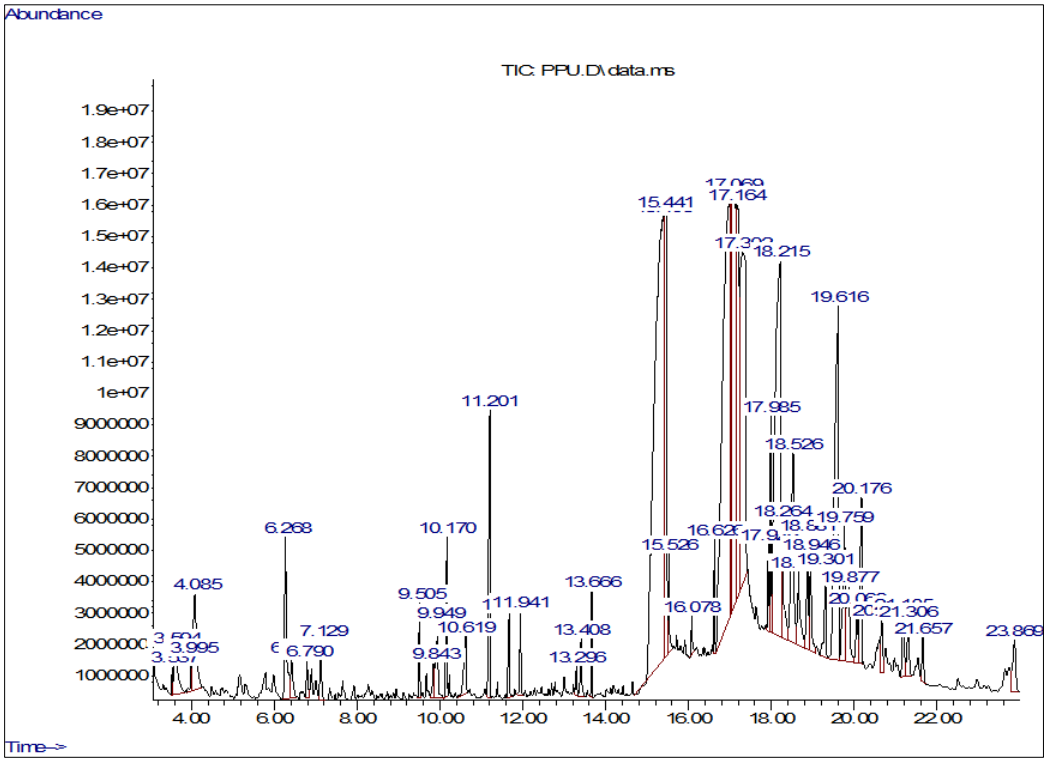


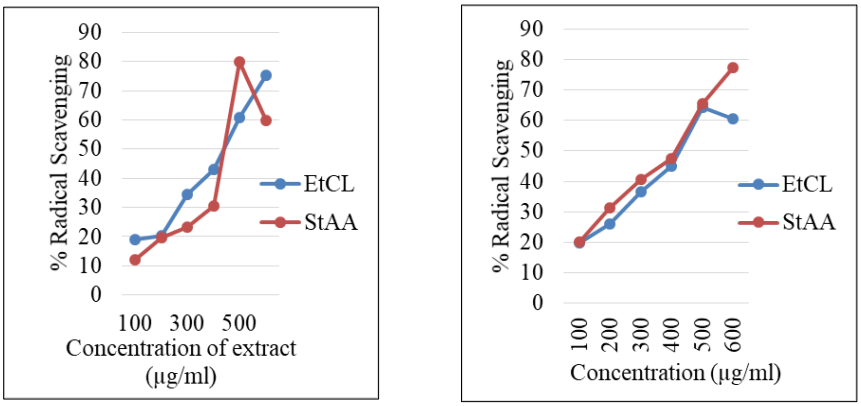

**Figure 1** Chromatogram of phytochemical constituents identified by GC-MS analysis of Ethanol Seed extracts of *Celosia leptostachya*

Oxidation reaction is an essential reaction in living cells that is associated with cellular energy production. Free radicals are often generated and accumulated during this reaction process result in oxidative stress [22]. There has been growing interest in oxidative stress and specific human diseases which can be prevented by wide range of plant derived antioxidants [23]. Antioxidants are agents that can prevent or slow oxidative damage to cells caused by free radicals and other unstable molecules [24]. *Invitro* antioxidant studies using DPPH and NO radical scavenging indicated that the ethanol extract of *Celosia leptostachya* leaves exhibited > 50 % inhibition at concentrations of 500 µg/ml and 600 µg/ml Figure 2. $IC_{50}$ values obtained from the linear regression analysis for NO showed that the extract and standard had 34.63 µg/ml and 22.45 µg/ml respectively.

**Table 1** Phytochemical Constituents of Ethanol Extracts of *Celosia leptostachya* Leaves

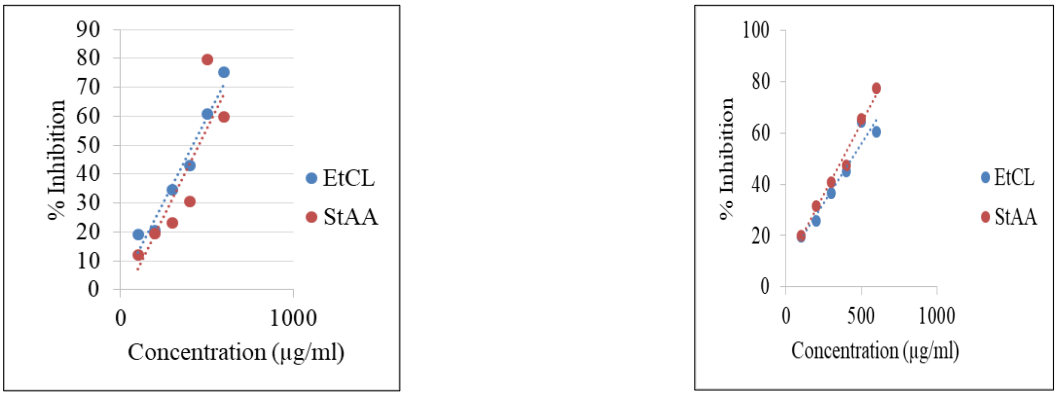
| S/N | Compound | RetentionTime (min) | Percentageof the total |
|---|---|---|---|
| 1 | Trans-4-amino Cyclohexanol | 3.537 | 0.099 |
| 2 | 5methyl Hex-1-ene | 3.594 | 0.804 |
| 3 | 2-Ethyl-1-butanol, trifluoroacetate | 3.995 | 0.189 |
| 4 | (Z)-Hept-2-ene | 4.085 | 1.476 |
| 5 | 2,4-(E,E)Nonadienal, | 6.268 | 1.431 |
| 6 | 3-Methoxyhex-1-ene | 6.416 | 0.388 |
| 7 | 3,8-dimethyl decane | 6.790 | 0.245 |
| 8 | Cyclohexen-1-one | 7.219 | 0.389 |
| 9 | 3,4,5-trimethyl cyclopent-1-one | 9.505 | 0.141 |
| 10 | 2,5-dimethyl cyclohexanone | 9.843 | 0.250 |
| 11 | 9-oxonanoic acid | 9.949 | 0.795 |
| 12 | -(3 butynyloxy)tetrahydro 2H-Pyran | 10.170 | 0.943 |
| 13 | 1, 2,3,4-trimethyl-1,4-Pentadiene | 10.619 | 0.658 |
| 14 | Diethyl Phthalate | 11.201 | 1.676 |
| 15 | morpholineBorane | 11.670 | 0.502 |
| 16 | 2(3H)-Furanone, 5-butyldihydro- | 11.941 | 0.421 |
| 17 | N-butyl Benzenesulfonamide | 13.296 | 0.171 |
| 18 | 4-Fluoroisocathinone | 13.408 | 0.390 |
| 19 | Octadecamethylcyclononasiloxane | 13.666 | 0.416 |
| 20 | n-Hexadecanoic acid | 15.403 | 17.971 |
| 21 | n-Hexadecanoic acid | 15.441 | 4.889 |
| 22 | n-Hexadecanoic acid | 15.526 | 0.387 |
| 23 | Heptadecanoic acid | 16.078 | 0.184 |
| 24 | tetradecamethylHexasiloxane | 16.626 | 0.469 |
| 25 | 9,12-Octadecadienoic acid (Z,Z)- | 16.992 | 12.938 |
| 26 | 9,12-Octadecadienoic acid (Z,Z)- | 17.024 | 2.330 |
| 27 | 9,12-Octadecadienoic acid (Z,Z)- | 17.069 | 6.443 |
| 28 | 9-Octadecenoic acid, (E)- | 17.164 | 5.397 |
| 29 | Octadecanoic acid | 17.302 | 7.474 |
| 30 | 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy) Tetrasiloxane | 17.929 | 0.418 |
| 31 | Hexadecanoic acid, pentyl ester | 17.985 | 1.019 |
| 32 | Bicyclo[2.2.1]heptane, 7,7-dimethy l-2-methylene- | 18.215 | 9.050 |
| 33 | cis-3-Butyl-4-vinyl-cyclopentene | 18.378 | 18.264 |
| 34 | cis-3-Butyl-4-vinyl-cyclopentene | 18.526 | 2.295 |
| 35 | cis-13-Eicosenoic acid | 18.659 | 0.856 |
| 36 | Eicosanoic acid | 18.881 | 0.914 |
| 37 | Octadecanoic acid, butyl ester | 18.946 | 0.777 |

| | | | |
|---|---|---|---|
| 38 | TetradecamethylHexasiloxane | 19.301 | 0.686 |
| 39 | Arachidonic acid | 19.616 | 5.839 |
| 40 | 13-Octadecenal, (Z)- | 19.759 | 1.495 |
| 41 | Methyl 6-cis,9-cis,11-trans-octadecatrienoate | 19.887 | 1.053 |
| 42 | Octadecanoic acid, pentyl ester | 20.069 | 0.467 |
| 43 | Cyclohexene, 3-methyl-6-(1- | 20.176 | 1.396 |
| 44 | methylethenyl)-, (3R-trans)- | 20.665 | 0.477 |
| 45 | 4,5-Octanediol, 3,6-dimethyl- | 21.185 | 0.574 |
| 46 | 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane | 21.306 | 0.515 |
| 47 | Carbonic acid, decylundecyl ester | 21.657 | 0.341 |
| 48 | Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene- | 23.869 | 0.694 |




A B

**Figure 2** Free Radical Scavenging Activity of Ethanol Extract of *Celosia leptostachya* leaves and Standard. A=DPPH and B= Nitric Oxide (NO ); Ethanol Extract *Celosia leptostachya* leaves (EtCL) and Ascorbic Acid standard (StAA represents mean ± SEM, n=3




C D

**Figure 3** Invitro Inhibition Activity for Determination of IC50 values. C=DPPH, D= NO Radical inhibition. Ethanol Extract of *Celosia leptostachya* leaves (EtCL) and Ascorbic Acid standard (StAA). Line of best fit were determined by least square linear regression analysis

The $IC_{50}$ values for DPPH showed 11.23 µg/ml and 16.78 µg/ml for the standard and extract respectively Figures 3-4. Antioxidants neutralize oxidative damage caused by free radical via several mechanisms which include scavenging, free radical chain reaction inhibitors, metal chelating agents and oxidative enzyme inhibitors to boost overall health [25, 26]. Studies conducted by [27] suggested that increasing the intake of dietary antioxidants helped to maintain the antioxidant status and normal physiological functions of the human body. Chlorogenic acid found in coffee has shown to have beneficial effect on cardiovascular diseases and decrease the risk of developing type II diabetes, oxidative damage linked to carcinogenesis, atherogenesis and aging [28,29,30].

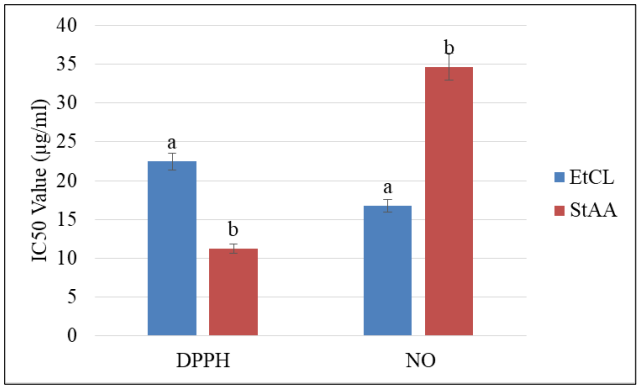
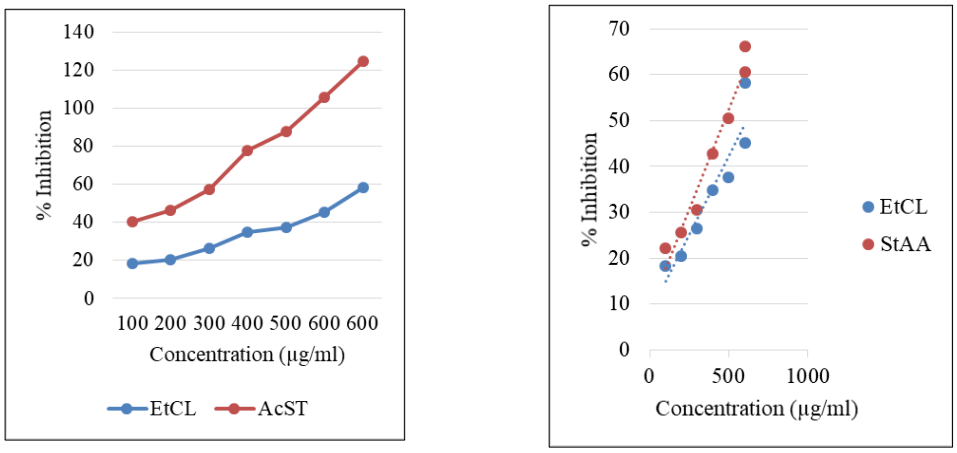



**Figure 4** IC50 Values of Ethanol Extracts of *Celosia leptostachya* leaves (EtCL) and Standard Ascorbic Acid (StAA).

Data represents mean ± SEM, n=3. Superscripts with different alphabets (a-b) are significantly different ($p< 0.05$, Kruskal-Wallis H test)

Alpha amylase is involved in the hydrolysis of starch into glucose which is absorbed by the body [31]. Alpha amylase inhibitors are considered as useful tools in control of hyperglycemia and diabetes mellitus [32]. In this study, alpha amylase inhibition showed an increase as the concentration of the extract increases. Inhibition pattern showed was similar to acarbose used as standard in Figure 5. $IC_{50\ values}$ of 5.02 µg/ml and 1.03 µg/ml were obtained for the extract and standard respectively using linear regression analysis Figures 5-6. The kinetic studies under non inhibitory condition gave Km and Vmax to be 182.92 µg/ml and 0.682 OD/min. under inhibitory conditions, these values were reduced to (76.34 µg/ml and 0.556 OD/min) Figure 6. The inhibition pattern showed noncompetitive model.The Ki value of 11.16 ug/ml from slope replot was obtained as shown in Figure 7. The inhibition mechanism gives an indication of ease of binding of the extract to the enzyme in order to decrease the rate of absorption of glucose thereby offering a strategy in treatment of hyperglycemia.




E F

**Figure 5** E=Alpha amylase inhibitory Activity, F= IC50 Values. EtCl =Ethanol Extract of *Celosia leptostachya* leaves AcST= Standard Acarbose. Values represents the mean ± SEM, n=3

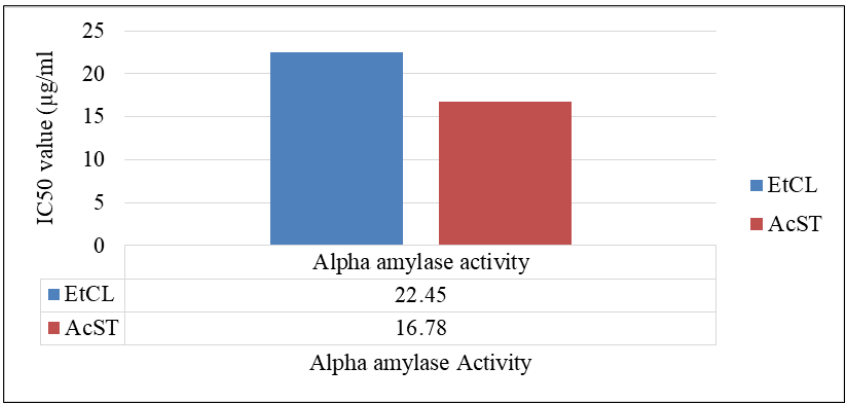
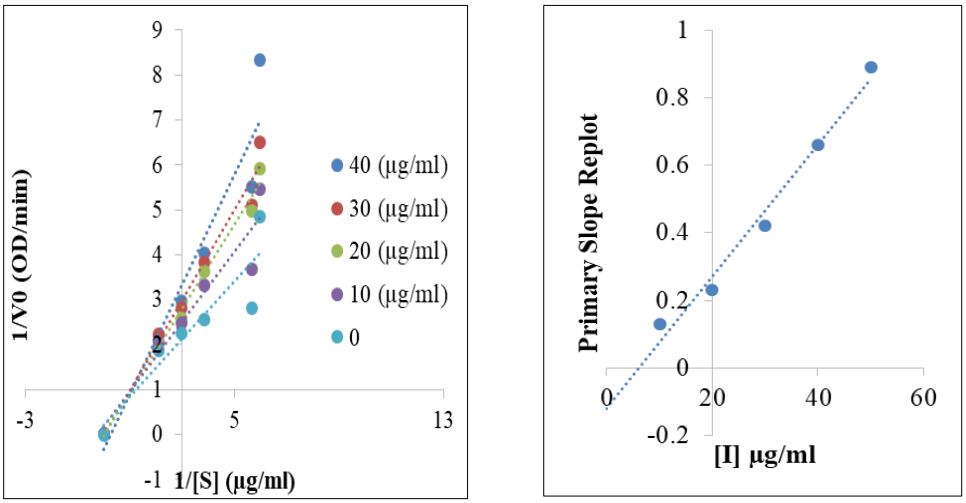



**Figure 6** IC50 Value of Ethanol Extracts of *Celosia leptostachya* leaves (EtCL)and Acarbose (AcST). Value represents the mean ± SEM (Standard Error of the Mean, n=3)




G H

**Figure 7** G=Lineweaver-Burk plot of Alpha-amylase Inhibition at Different Substrate Concentration [S] (10, 20, 30 and 40) µg/ml in absence (0) or presence of extract. H=Secondary replot of slope against inhibitor concentrations to calculate for inhibition constant (Ki). Line of best fit were determined by least square linear regression analysis

## 4. Conclusion

From this Study, it can be concluded that ethanol seed extract of *Celosia leptostachya* leaves contained essential phytochemical constituents that can be explored for pharmacological and broad-spectrum bioactivity. The exhibited extract exhibited antioxidant and enzyme inhibitory properties which are attributed to the presence of essential phytochemical components. The kinetic mechanism of alpha amylase inhibition showed noncompetitive model which can be explored for development of therapy used for the treatment of hyperglycemia and associated diseases.

## Compliance with ethical standards

### *Acknowledgments*

On behalf of the authors, the corresponding author would like to thank the Rivers State University for providing the platform for which this research was carried out.

### *Disclosure of conflict of interest*

On behalf of all authors, the corresponding author declares that there is no conflict of interest.

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