

Evaluation of Antioxidant Activities of *Celosia trigyna* (Linn) Extracts African Extinction Vegetable

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Abstract: *Celosia trigyna* recognized as a known ancient vegetable unfortunately unpopular in this modern age. In this study, antioxidant activities of *Celosia trigyna* leaves were evaluated in crude extract (methanol) and different fractions; chloroform, hexane, methanol and ethylacetate. Antioxidant evaluation were established using total antioxidant activity (phosphomolybdeum) and antiradical scavenging activity (2,2-Diphenyl-1-Picrylhydrazyl (DPPH)) and Hydrogen Peroxide (H₂O₂) methods. *C. trigyna* leaves showed the highest values of Total phenolics content (TP) (0.162mg, Rutin equivalent per g methanol fraction) while the lowest value of TP (0.092mg ± 0.001 GAEG-1) was recorded in the chloroform fraction. Ethylacetate fraction of *C. trigyna* leave extract was highest (0.192 ± 0.003mg GAEG-1) in Total Antioxidant Activity. Interestingly the *C. trigyna* methanol fraction exhibited the highest percentage inhibition for both DPPH and H₂O₂ scavenging activities of 89.22% and 88.24% respectively at concentration 0.6mg/ml. All the fractions including the *C. trigyna* crude methanol extract showed strong scavenging activity on DPPH as the concentration increases while the scavenging activity of the *C. trigyna* leaves methanol extract and fractions were high at 0.6mg/ml concentration. *C. trigyna* is presented as an alternative natural antioxidant to synthetic antioxidants used in foods and pharmaceutical industries.

Keywords: *Celosia trigyna*, Methanol Extract, Antioxidant, Vegetable

1. Introduction

Recently, research focus on plants has incredibly risen all over the world and quite a several plants are discovered to have medicinal advantages especially for humans [1]. Developing countries around the world invest immensely on traditional herbs in the treatment of diseases. Since civilization began, Human beings within their Habitat found remedies to ailments and adopted several strategies varying climate, socio-cultural, floral, and phytogeographic characteristics. Traditionally several methods are invoked to get rid of diseases and ailments which include the use of

plants as herbs for prevention and cure [2]. Several medicinal plants are found around the world, this is the plant with the potency of healing; which has therapeutic effects or foundational compounds that can be synthesized for useful drugs [3]. Human has depended largely on medicinal drugs in past centuries. Though a great number of research works have been done on numerous medicinal plants especially from the *Amaranthus*. This study is aimed at evaluating the antioxidant activities of the crude extract of *Celosia trigyna* L leaf (extinction vegetable) and its fractionated extracts.

The *Celosia* species is an edible plant belonging to the

Amaranthaceae, it is ornamental with a small genus. Its name *Celosia* was derived from the Greek word for 'burned' which is 'Kelos', signifying it flamelike flowerhead [4]. *C. trigyna* L is an erect annual herb of about 120(-180) cm tall; has a simple or branched stem, grooved, glabrous, or with few hairs, usually pink to silver color. The leaves are alternate, simple, without stipule petiole up to 5(-8) cm long. *Celosia* plants occur has almost fifty (50) species in the region of tropical and subtropical, *C. trigyna* L in the tropical is said to be the most widespread. Vegetables such as *Celosia globosa*, *C. isertii*, and *C. leptostachya* are often confused with *Celosia trigyna*. Among plants of this species, *C. trigyna* though not well known in this present age is a wild type of *Celosia* found almost throughout tropical Africa, South Africa, and Southern Arabia. It is known as a weed called aje fo awo meaning eat and break the plate, it is a vegetable that is common among the old people in the Southern part of Nigeria.

Generally, most of the *Celosia* species are known in the treatment of different ailments such as diarrhea, disinfectant, inflammation, bleeding nose hematological, piles, treating cold and gynecological disorders according to traditional practitioner consulted. He further disclosed that when crushed, leaves can be used in the treatment of chest trouble and stomach aches. The *C. trigyna* leaves have been using to conclude the several medicinal preparations using in the treatment of women's disorder diseases, including ovarian troubles and treatment of wounds.

Antioxidant activity is a very important pharmacological property. The property of the plant is responsible for the functions it performs such as anti-aging, anti-carcinogen, antimutagenicity, and so on. Superoxide, hydroxyl, and nitric oxide radicals are reactive oxygen and nitrogen which are also referred to as the most important free radicals of the body. The consequence of cellular and metabolic activities generates these free radicals. Excessive production and leakage from their site cause degeneration of the body cells and they appear to be the major contributor to aging and diseases. These free radicals also contain unpaired electrons; thus, making them unstable, having an affinity for electrons from other substances to get them neutralized. This firstly stabilizes the radicals but also generates another radical in this process causing more chain reactions which can occur within a few seconds [5]. Synthetic and Natural antioxidants are the two known categories. The synthetic type is made of phenolic compounds with alkyl substitution while the natural antioxidant can either be made of phenolic, nitrogen, and carotenoids or ascorbic. Those of phenolic are in the form of tocopherols, flavonoids, and phenolic acid while nitrogen compounds are alkaloids, chlorophyll derivatives, amino acids, and amines. Plant in nature contains natural antioxidants because of their exposure to ultraviolet light during the day leading to the synthesis of various free radicals. These free radicals have a built-in system that inhibits cellular damage which may lead to dying and weathering of the plant [6].

Vitamins Such as A, C, E which are vitamin Anti-

oxidants are needful to be included in the diet because they are not naturally produced by the human body, folic and beta carotene are also good anti-oxidation which are part of human daily food intakes. Vitamin A (retinol and carotenes) is very useful in the improvement of the immune system, repair of wore out tissues, eye healthiness, and control human cholesterol level. Vitamin C protects the skin from damage, infections, and promotes good iron absorption. Vitamin E helps healthy blood vessels, protecting the body membrane, and improving skin conditions. In regards to the protection against free radicals, beta-carotene is the best [7]. All of these vitamins are most commonly found in oranges, colored vegetables such as spinach, carrot. With the aid of plant antioxidants, free radicals are converted into health-promoting less reactive species which in turn prevent degenerative diseases. Due to the latest discoveries of defects from synthetic antioxidants, the biochemical function of the plants with naturally occurring antioxidants is being focused. The human body can then be protected from oxidative stress, free radicals and associated diseases through substances derived from the plants which are a good source of natural antioxidative of the most important natural antioxidants are the phenolic compounds which reduce agents and activators of an antioxidant defense-enzyme system that suppress the damages from radicals in the biological system. Thus, this study is purposed to evaluate the new potentials of the natural anti-oxidant in *Celosia trigyna* L., which will be contributing to the antioxidant activity of the vegetable extract for the development of new drugs and food preservatives.

2. Materials and Methods

2.1. Plant Collection

Trigyna L. leaves were collected at the back of the faculty of Sciences, the Polytechnic, Ibadan, North local Government Area, Oyo State, Nigeria. The geographical location of the leaves collection area is approximately longitude 07°25' 36.61"N and 07°27' 22.7' N and latitude 03°52' and 03°53' 45.81"E [8]. Identification authentication of *C. trigyna* L was done at Herbarium section of Forest Research Institute of Nigeria with FRI NO 111896.

2.2. Preparation of Crude Extract

The leaves *Celosia trigyna* L. were pulverized into a coarse powder. 766.9g of the powder leaves were soaked in 5liters of 95% methanol for 72hours, the extract decanted and filtered. A rewash of the residues with methanol was carried out and the two filtrates collected were further filtered using Whatman filter paper (1mm). The filtrate collected was then concentrated using a rotary evaporator that was set at 40°C. This is the Crude Methanol Extract of *Celosia trigyna* plant leaves, which was further concentrated using a vacuum oven set at 40°C with a pressure of 700mmHg, placed in a glass bottle, and preserved for onward use.

2.3. Fractionation of *Celosia trigyna* L. Leaves Methanol Extract Using Vacuum Liquid Chromatography (VLC)

Fractionation of *Celosia trigyna* L. leaves methanol extract was achieved by modifying the method of Okwuchi (2015) [9]. *Celosia trigyna* L. leaves methanol extract (12 g) was dissolved in 120 ml of methanol to make it easy to mix with silica gel powder. 100g of silica gel was weighed and placed into the sintered glass funnel compressed under pressure from the vacuum pump and then rinsed with n-hexane, drained dried under pressure before the crude extract mixture was packed and pressed under pressure. The Crude Method Extract of *C. trigyna* (CMECT) was pre-adsorbed, dried, and used to prepare the extract bed. The solvents were added from the less polar to the more and drained until a very clear solvent was observed on each elution. The fractions were concentrated and the remaining solvent in the concentrates was further allowed to dry and the contents were then stored in glass containers and stored at 4°C until ready for use. The fractions obtained were a fraction of the hexane (HFCT), chloroform fraction (CFCT), ethyl acetate fraction (EFCT) and methanol fraction (MFCT). The vacuum liquid chromatography and thin-layer chromatography techniques were used for fractionation. The different fractions (HFCT, CFCT, EFCT, and MFCT) obtained from the CMECT of *Celosia trigyna* leaves were spotted on a pre-coated TLC plate of the different solvent mobile phase.

2.4. Total Phenolic Content Estimation

The total phenolic content of the *Celosia trigyna* leaves crude and fraction extracts were determined by spectrophotometric method according to Folin – Ciocalteu method using gallic acid to set up the standard curve. 0.25ml of the extract was mixed with 0.5ml Folin-C phenol reagent. After 5minutes, 5ml of 7% Na₂CO₃ solution was added to the mixture, followed by the addition of 65ml distilled water. The mixture was thoroughly mixed, allowed 90minutes incubation at 25°C and thereafter the absorbance was reading at 750nm. The total phenolic content of *C. trigyna* leaves crude and fraction extracts were evaluated from a gallic acid standard curve expressed as GAE meaning Gallic Acid Equivalent, ml/g of extract. Blank is distilled water and the values were in triplicates [10].

2.5. Determination of Antioxidant Activities

The antioxidant activities of *Celosia trigyna* leave crude and fraction extracts were established using three (3) different antioxidant assays noting that one method cannot be sufficient to prove the antioxidant efficiency of plant products [11].

2.5.1. DPPH Radical Scavenging Activity

The radical solution was prepared by dissolving 2.5mg of DPPH in 100ml of methanol (0.025g/l) solution. Different concentrations of the *C. trigyna* leaves stock extract solution (1mg/ml) were added to 3.9ml of DPPH solution and the reactants incubated at 25°C room temperature for 30 minutes.

In place of an extract, the Ascorbic Acid solution (a positive control) was used as standard. The mixture was shaken and the solution was allowed to stand in the dark at room temperature for 35 minutes. Free radical scavenging activity was calculated from absorbance values at 517 nm. Blank was also measured which was distilled water.

$$\% \text{ Inhibition} = \frac{\text{Abs of Control} - \text{Abs of extract}}{\text{Abs of Control}} \times 100$$

2.5.2. Total Antioxidant Activity

The total antioxidant activity of the *Celosia trigyna* leaves crude and fraction extracts were assessed spectrophotometrically by the phosphomolybdenum method [12]. 1ml of *C. trigyna* leaves samples was mixed separately with 3ml of reagent solution (28mM Sodium Phosphate buffer, 0.6M H₂O₄ and 4mM ammonium molybdate); the blank solution contained 4ml of reagent solution only. The mixtures were passed through incubation for 90mins at 95°C. After, the absorbance was measured at 675nm after the mixture had cooled to room temperature. Total antioxidant activity was expressed as Ascorbic acid equivalent.

2.5.3. Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide (H₂O₂) scavenging activity of *C. trigyna* leaves crude and fraction extracts was determined by replacement titration method with shift mullification. Aliquot of 2ml of 1mM H₂O₂ and 1ml of various concentrations of the extracts were mixed, then added 2 drops of 3% ammonium molybdate, 7 ml of 1.8mM potassium iodide, 10ml of 0.2 MH₂SO₄, and 2 drops of 1% Starch indicator. The mixtures from the *C. trigyna* leaves samples were titrated separately with 0.5mM sodium thiosulphate until the disappearance of blue color. Percentage of scavenging of H₂O₂ was calculated as:

$$\frac{\text{Abs control} - \text{Abs of sample}}{\text{Abs of Control}} \times 100$$

$$\text{Inhibition (\%)} = V_0 - V_1 / V_0 \times 100$$

Where V₀ and V₁ were the volume of sodium thiosulphate used for the titration of blank and the sample extract respectively. IC₅₀ (mg/ml), which denotes effective concentration yielding 50% inhibition of H₂O₂ radicals was also calculated [13].

3. Results and Discussion

The percentage yield of *Celosia trigyna* L leaves extract (methanol crude extract) was 10.65%. The total phenolic contents of the leaves of *C. trigyna* methanolic extract is 0.144mg ± 0.005 GAEg⁻¹, hexane fraction (0.094±0.003 GAEg⁻¹), chloroform fraction (0.092±0.001 GAEg⁻¹), ethyl acetate fraction (0.107±0.002 GAEg⁻¹) and methanol fraction of *C. trigyna* is 0.162±0.003 GAEg⁻¹ as shown in table 1. This shows that naturally occurring antioxidants are primarily phenolics that are from all parts of plants especially from fruits and vegetables [14-16]. The DPPH scavenging

activities of *C. trigyna* L leaves methanol crude extract and its fractions were presented in table 2. The DPPH scavenging activities of the extract and fractions were compared with Ascorbic acid. Inhibition Percentage of the *C. trigyna* leaf extract and its fractions on DPPH Scavenging activity were present in Table 3 where methanol fraction of *C. trigyna* (MFCT) has the highest Percentage Inhibition on DPPH Scavenging Activity at 0.6mg/ml relative to the standard. In vitro condition, DPPH is considered as a stable free radical where it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduced ability of DPPH radical Scavenging was determined by the decrease in its absorbance at 517nm and by the disappearance of purple color to a new color, yellow which is induced by antioxidants. The coloration indicates the scavenging potential of the antioxidant compound in the extract. The different fraction of the enable extract at various concentrations induced a rapid decrease in the optical density.

Total Antioxidant activities can be defined as the measure of the number of free radicals scavenged. The Total Antioxidant Activity was highest (0.192 ± 0.003 mg GAEg⁻¹) in ethyl acetate fraction of *C. trigyna* leave extract followed by methanol fraction of *C. trigyna* (0.141 ± 0.001 mg GAEg⁻¹), leaves crude methanol extract of *C. trigyna* (0.093 ± 0.003 mg GAEg⁻¹), chloroform fraction of *C. trigyna* (0.051 ± 0.012 mg GAEg⁻¹) while hexane fraction of *C. trigyna* (0.010 ± 0.001 mg GAEg⁻¹) has the least total antioxidant activity (Table 4). The Hydrogen Peroxide Scavenging Activity of the *C. trigyna* leaves methanol extract and its fractions and Percentage Inhibition of *C. trigyna* leaves extract and its extract fractions on hydrogen period (H₂O₂) were presented in Tables 5 and 6 respectively. The *C. trigyna* methanol fraction exhibited the highest percentage inhibition for H₂O₂ scavenging activity of 88.24% at concentration 0.6 mg/ml whereas the chloroform fraction exhibited the lowest percentage inhibition for H₂O₂ scavenging activity of 12.50% at concentration 1.0mg/ml.

Table 1. Total Phenolics Content (GAEmg/g) of the *Celosia trigyna* leaves extract and its fractions.

<i>Celosia trigyna</i> leaf extract	GAEmg/g
CMECT	0.144±0.005
HFCT	0.094±0.003
CFCT	0.092±0.001
EFCT	0.107±0.002
MFCT	0.162±0.003

Table 2. DPPH scavenging assay for *Celosia trigyna* leaves methanol crude extract and its fractions UV Absorbance Readings (Optical Density).

Concentration	0.2. mg/ml	0.4 mg/ml	0.6 mg/ml	0.8 mg/ml	1.0 mg/ml
Standard Ascorbic acid	0.166	0.117	0.075	0.048	0.026
CMECF	0.562	0.428	0.368	0.344	0.329
HFCT	0.625	0.552	0.537	0.531	0.521
CFCT	0.425	0.422	0.420	0.413	0.335
EFCT	0.386	0.361	0.351	0.276	0.243
MFCT	0.312	0.283	0.279	0.270	0.252

Blank (water) = 0.047 Control (2ml DPPH = 0.878).

Table 3. Inhibition Percentage of the *Celosia trigyna* leaves methanol crude extract and its fractions on DPPH Scavenging activity.

Concentration	0.2. mg/ml	0.4 mg/ml	0.6 mg/ml	0.8 mg/ml	1.0 mg/ml
Std. Ascorbic Acid	81.09%	86.67%	91.46%	94.53%	97.04%
CMECT	35.99%	51.25%	58.09%	60.82%	62.53%
HFCT	28.82%	37.13%	38.84%	39.52%	40.66%
CFCT	51.59%	51.94%	52.16%	52.96%	61.85%
EFCT	56.04%	58.88%	60.02%	68.56%	72.32%
MFCT	64.46%	67.77%	89.22%	69.25%	71.30%

*MFCT has the highest Percentage Inhibition on DPPH Scavenging Activity at 0.6mg/ml relative to standard.

Table 4. Total Antioxidant Activity (TAA) (GAEmg/g) of the *Celosia trigyna* leaves methanol crude extract and its fractions.

<i>Celosia trigyna</i> leaves extract	GAEmg/g
CMECT	0.093±0.003
HFCT	0.010±0.001
CFCT	0.051±0.012
EFCT	0.192±0.003
MFCT	0.141±0.001

EFCT has the highest TAA among all the fractions of CT

Table 5. Hydrogen Peroxide Scavenging Activity of the *Celosia trigyna* leaves methanol extract and its fractions.

Concentration	0.2mg/ml	0.4mg/ml	0.6mg/ml	0.8mg/ml	1.0mg/ml
Blank	2.10ml	2.90ml	5.10ml	2.55ml	1.80ml
CMECT	0.70ml	0.90ml	1.30ml	1.30ml	1.80ml
HFCT	0.10ml	0.80ml	1.20ml	0.80ml	0.60ml
CFCT	0.70ml	0.90ml	0.60ml	1.90ml	1.70ml
EFCT	0.60ml	0.80ml	1.10ml	0.90ml	0.80ml
MFCT	0.40ml	0.50ml	0.60ml	1.20ml	0.9ml

Table 6. Percentage Inhibition of *Celosia trigyna* leaves extract and its extract fractions on hydrogen period (H₂O₂).

Extracts Concentration	0.2mg/ml	0.4mg/ml	0.6mg/ml	0.8mg/ml	1.0mg/ml
CMECT	66.67%	68.97%	74.51%	49.02%	-
HFCT	95.24%	72.41%	76.47%	68.63%	66.67%
CFCT	66.67%	68.97%	88.23%	25.49%	12.50%
EFCT	71.43%	72.41%	78.43%	64.71%	55.56%
MFCT	80.95%	86.21%	88.24%	52.94%	50.00%

Key:

CMECT- Crude Method Extract of *Celosia trigyna* HFCT- Hexane fraction of *Celosia trigyna* CFCT- Chloroform fraction of *Celosia trigyna*

EFCT- Ethyl acetate fraction of *Celosia trigyna* MFCT- Methanol fraction of *Celosia trigyna*

4. Conclusion

In the study Tin Layer Chromatography (TLC) investigation of *C. trigyna* L leaves extracts revealed that there is high hope of exploring more antioxidant compounds for therapeutic and drug manufacturing following the trend of all the antioxidant assays and in the food industries, *C. trigyna* L leaves showed a high inhibitory property both in the DPPH and H₂O₂ scavenging activities. Moreover, Total Phenolic content and Total Antioxidant determination showed that there is hope for this wild spinach to be explored deeper for use. These indications also show that the leaves of *C. trigyna* L have beneficial health effects in humans as they

help in the treatment of sores and boils costal pains, heart complaints, stomach ache, urethral disorder, and chest pains. The *Celosia trigyna* L leaf is not only used in treating ailment but also very well in soup making, in that the Yoruba call it “aje fo awo, meaning eat and break plate”. Interestingly finding naturally occurring antioxidants has increased to replace synthetic antioxidants which are toxic and carcinogenic. The study also established that phenolic compounds contribute greatly to the antioxidant activity of *C. trigyna* L leaf extracts.

5. Recommendation

Further studies on *C. trigyna* L leaf extracts are recommended to be carried out to isolate and purify the active compounds useful in the pharmaceutical and food industries because the plant has shown various compounds on thin-layer chromatography (TLC) plates. The promising compounds separated on TLC plates may be responsible for *C. trigyna* L leaf traditional treatment of diseases including ovarian troubles, excessive menstruation, women's disorders, cancer, and so on.

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