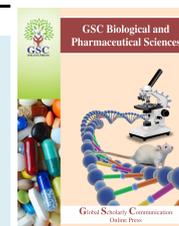


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(RESEARCH ARTICLE)



Phytochemicals and chelating properties in extract of *Celosia trigyna* inhibits xanthine oxidase *in vitro*

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Abstract

This study is designed to investigate the inhibitory effect of *Celosia trigyna* against xanthine oxidase. Total flavonoid, phenol, vitamin C were evaluated using standard methods and inhibitory activity of *Celosia trigyna* extract on xanthine oxidase (XO) activity *in vitro* and chelating properties of extract against Iron (II) were also carried out. The results showed that the extract contained high content of flavonoids, phenolic acid and vitamin C that are beneficial to health. The extract inhibited XO in a dose-dependent manner. The extract also chelated Fe²⁺ in a dose-dependent manner. *Celosia trigyna* extract could be a promising nutraceutical for preventing and managing hyperuricaemia due to its ability to inhibit XO; this activity is attributed to the combined effect of its phytochemicals and chelating properties.

Keywords: *Celosia trigyna*; Total flavonoid; Vitamin C; Phenolic acid; Xanthine oxidase

1. Introduction

Many diseases, or at least symptoms of diseases, arise from a deficiency or excess of a specific metabolite in the body. For an example an excess of a particular metabolite that produces a disease state is the excess of uric acid which can lead to gout. Inhibition of XO decreases the uric acid levels, and results in an antihyperuricemic effect. Allopurinol, first synthesised as a potential anticancer agent, is nowadays a clinically useful xanthine oxidase inhibitor used in the treatment of gout. Gouty arthritis or uric acid nephrolithiasis is the elevated concentration of uric acid, otherwise known as hyperuricaemia, leads to the deposition of monosodium urate monohydrate crystals in tissue, especially joints [1].

Xanthine oxidase is a form of xanthine oxidoreductase, a type of enzyme that generates excessive production of reactive oxygen species, free radical which are linked to degenerative diseases such as cancer and damage of the hepatic system [2]. However, there are growing evidence that the inhibition of xanthine oxidase activity could play a protective role in some disease conditions (gout). The consumption of medicinal plants and use of dietary antioxidant and bioactive compound from plant and plant extracts have been established for the treatment or management of some diseases. *Celosia trigyna* contain active components which have been reported to exert multiple biological effect including antioxidant and free radical scavenging activities [3] however, to the best of our knowledge, the protective properties of *Celosia trigyna* against gout has not been reported.

The ability of XO to generate superoxide and hydrogen peroxide in the presence of molecular oxygen, hypoxanthine and xanthine is well known. On the other hand, the increased formation of O₂⁻ in several pathological conditions could lead to the release of iron from ferritin. Free iron is liable to catalyse the conversion of superoxide into the extremely

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reactive and damaging hydroxyl radical, by Haber-Weiss or Fenton driven reactions. This radical is one of the most potent oxidants known by its capacity to damage key cell structures [1].

Celosia trigyna leaves are consumed as vegetables, finely cut in soups, stews and sauces. The slightly bitter leaves are popular amongst the Yoruba people in southern Nigeria where the plant is known as “Aje fo wo”. The plant is used in traditional medicine. In Sierra Leone it is used for the treatment of heart complaint whereas in Northern Nigeria it is used to treat postular skin eruption. In Ghana it is applied to sores and boils [4, 5] Pulped leaves are used to treat coastal pains, chest troubles, stomach ache and urethral disorders. The plant is included in several medicinal preparations used to treat women’s disorder and diseases, including ovarian troubles in DR Congo and excessive menstruation in Ethiopia. The leaves and flowers are used to treat diarrhoea. The plant is also used for treatment of guinea worm infection, the leaves together with alligator pepper seeds (*Amomum Subulatum*) are ground for this purpose. Seven incisions are made on the affected part and the mixture rubbed in [4, 5].

Chronic allopurinol administration for the inhibition of XO is clinically effective against the hyperuricemia associated with gout, but undesirable side effects have prompted efforts to isolate or synthesise other types of XO inhibitors. Hence this study is designed to investigate the inhibitory effect of *Celosia trigyna* against xanthine oxidase.

2. Material and methods

2.1. Chemicals

Iron (II) chloride tetrahydrate, Sodium chloride, Ethanol, Tris (hydroxymethyl amino methane), 1, 10-phenanthroline, thiourea xanthine were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Samples collection and preparation

Leaves of *Celosia trigyna* were harvested from the plant in Amassoma, Southern Ijaw Local Government Area of Bayelsa State, Nigeria, in February, 2018. The leaves were botanically identified at the Department of Botany, Niger Delta University, Nigeria. Thereafter, they were shade-dried for two weeks. The leaves were later milled into fine particle size and (20 g) extracted with 200 ml of 80% ethanol at 80 °C for 3 h. The extract was concentrated to dryness under reduced pressure in a rotary evaporator to yield ethanol extract which was stored in the freezer for further analysis.

2.3. Experimental animals

Adult male Wistar strain albino rats weighing 150–200 g were procured from the experimental animal breeding unit of Department of Pharmacology, Niger Delta University, Nigeria. The animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health (USA) [6]. The rats were kept in a cage to acclimatize for 7 days, during which they were maintained at room temperature under the laboratory conditions and were fed with standard diet and water *ad libitum*.

2.4. Preparation of liver homogenate for XO inhibition assay

The liver tissue homogenate for XO assay was prepared following the method described by Nakamura et al., 2014. The liver was rapidly excised after decapitation of the rats under mild ether anesthesia. The liver was washed in cold 0.15 mol/L KCl, and blotted dry. Then a portion of 1 g of liver tissue was homogenized in 9 volumes of ice-cold 50 mmol/L Tris-HCl buffer (pH 7.4) containing 1 mmol/L ethylene diamine tetraacetic acid (EDTA). A portion of the homogenate was centrifuged for 10 min at 1400 × *g* to yield a supernatant that was used for the XO.

2.5. Xanthine oxidase inhibition assay

The ability of the extract to inhibit XO was determined following the method reported by Umamaheswari et al. [7] with slight modification. The reaction mixture consisted of 300 uL of 50 mmol/L sodium phosphate buffer (pH 7.5), 100 uL of the extract at different concentrations (1, 3, 5 and 7 mg/mL) in dimethyl sulphoxide (DMSO), 100 uL of freshly prepared tissue enzyme preparation and 100 uL of distilled water. The assay mixture was preincubated at 37 °C for 15 min. Then, 200 uL of 0.15 mmol/L of xanthine solution (substrate) was added to the mixture which was incubated at 37 °C for 30 min; this was followed by the addition of 200 uL of 0.5 mol/L HCl to terminate the reaction. A reference test containing 100 uL of DMSO instead of the extract was carried out in order to obtain the maximum uric acid formation. The absorbance was measured at 295 nm on a UV/VIS spectrophotometer against a blank prepared in

the same way except that the enzyme solution was replaced with the phosphate buffer. One unit (U) of this enzyme is defined as the amount of enzyme required to form 1 mmol of uric acid per min at the reaction conditions. The percentage XO inhibitory activity of the extract was calculated thus: where $A_{295\text{reference}}$ is the reference without the extract, and $A_{295\text{sample}}$ is the absorbance of test containing the extract.

$$\% \text{ XO inhibition} = (A_{295\text{reference}} - A_{295\text{sample}}) / A_{295\text{reference}} \times 100$$

2.6. Total phenol

The total phenol content of plant extract was estimated using the Folin–Ciocalteu reagent method as described by Singleton et al. [8] and Demiray et al. [9]. The plant sample (100 mg mL⁻¹, 1.0 mL) was mixed thoroughly with 5 mL Folin–Ciocalteu reagent (diluted ten-fold) and after 5 minutes, 4.0 mL of sodium carbonate (0.7 M) was added and the mixture was allowed to stand for 1 h with intermittent shaking; for color development. The absorbance was measured at 765 nm in a spectrophotometer against a blank. The blank solution contained the solvent used to dissolve the plant extract. Gallic acid was used as a standard. Total phenol contained in Plant extract was calculated as gallic acid equivalents (GAE).

2.7. Total flavonoids

The total flavonoid content of plant extract was determined using a colorimetric method described by Zhishen et al. [10] with slight modifications. Plant (100 mg) was dissolved in 10 mL of 80% methanol and the resultant homogenous mixture was allowed to stand for 20 minutes at room temperature. This was followed by filtration through Whatman filter paper (No 42). An aliquot of 0.4 mL of the filtrate was mixed with 0.6 mL distilled water and 5% NaNO₂ solution (0.06 mL). The mixture was allowed to stand for 5 minutes at room temperature. After 6 minutes 10% AlCl₃ solution (0.06 mL) was added to the mixture. This was immediately followed by the addition of 1M NaOH (0.4 mL) and 0.45 mL distilled water to the mixture and allowed to stand for another 30 minutes. Absorbance of the mixture was determined at 510 nm. Quercetin calibration curve was prepared at the same wavelength (510 nm) and used for the quantification of total flavonoid content.

2.8. Total vitamin C

Ascorbic acid was determined according to the method of Klein and Perry [11]. The dried ethanolic extract (100 mg) was extracted with 10 ml of 1% metaphosphoric acid for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml of 2, 6-dichlorophenolindophenol and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (1– 5 mg/ml) and the results were expressed as mg of ascorbic acid/g of extract.

2.9. Fe²⁺chelation assay

The Fe²⁺chelating ability of the extract was determined using a modified method of Minotti and Aust [12] with a slight modification by Puntel et al. [13]. Freshly prepared 500 μM FeSO₄ (150 μl) was added to a reaction mixture containing 168 μl 0.1M Tris-HCl (pH 7.4), 218 μl saline and the extract (0–25 μl). The reaction mixture was incubated for 5 min, before the addition of 13 μl 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the JENWAY UV-Visible spectrophotometer. The Fe (II) chelating ability was subsequently calculated as % Fe²⁺ chelation = $(\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}) / \text{ABS}_{\text{control}} \times 100\%$.

2.10. Statistical Analysis

Statistical analysis was evaluated using SPSS.

3. Results and discussion

Table 1 Inhibition of xanthine oxidase and chelation of iron (II) by the extract of *Celosia trigyna*

(mg/ml)	Xanthine oxidase inhibition (%)	(%) inhibition Fe ²⁺
1	40.5 ± 0.55	27.1 ± 0.99
3	49.1 ± 1.05	32.66 ± 1.22
5	57.3 ± 2.09	43.2 ± 3.1
7	69.4 ± 0.76	60.42 ± 2.09

Each experiment was repeated three times. Results have been expressed as mean ± standard deviation

Table 2 Bioactive components of *Celosia trigyna* extract

Phytochemical	Concentration/g extract
Flavonoid	4.72 ± 0.97 mgQE/g extract
Vitamin C	2.63 ± 1.45 mgAAE/g extract
Phenol	2.51 ± 0.21 mgGAE/g extract

Each experiment was repeated three times. Results have been expressed as mean ± standard deviation

Phenolic compounds are among the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants as they play an important role in growth, reproduction, and protection against pathogens and predators. Phenolic compounds exhibit a wide range of physiological properties and have been associated with the health benefits [14]. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations. The role of antioxidants in preventing oxygen radical- and hydrogen peroxide-induced cytotoxicity and tissue damage is becoming increasingly recognized [15]. The results of the present study showed 2.51 ± 0.21 mgGAE/g extract phenolic content.

The result of the present study revealed 2.63 ± 1.45 mgAAE/g extract of vitamin C. Ascorbic acid includes two compounds with antioxidant activity: L-ascorbic acid and L-dehydroascorbic acid which are both absorbed through the gastrointestinal tract and can be interchanged enzymatically in vivo. Ascorbic acid is effective in scavenging the superoxide radical anion, hydrogen peroxide, hydroxyl radical, singlet oxygen and reactive nitrogen oxide [16].

The value of the flavonoid content was 4.72 ± 0.97 mg QE/g extract. Flavonoid antioxidant properties are conferred on by the phenolic hydroxyl groups attached to ring structures and they can act as reducing agents, hydrogen donors, singlet oxygen quenchers, superoxide radical scavengers and even as metal chelators. They also activate antioxidant enzymes, reduce α -tocopherol radicals (tocopheroxyls), inhibit oxidases, mitigate nitrosative stress, and increase levels of uric acid and low molecular weight molecules. Some of the most important flavonoids are catechin, catechin-gallate, quercetin and kaempferol [17].

Hydrogen peroxide easily diffuses across the plasma membrane. Hydrogen peroxide is also produced by xanthine oxidase, amino acid oxidase, and NAD(P)H oxidase and in peroxisomes by consumption of molecular oxygen in metabolic reactions. In a succession of reactions called Haber–Weiss and Fenton reactions, H₂O₂ can breakdown to •OH in the presence of transition metals like Fe²⁺ or Cu²⁺ [18]. To avert the dangerous effect of •OH phenols, flavonoids and vitamin C can chelate these transition metals thereby rendering them redox inactive. The chelating property of the extract (*Celosia trigyna*) show a dose dependent manner and at 7 mg/ml extract the chelated 60.42 ± 2.09 of redox metal (Fe²⁺).

Both allopurinol and oxypurinol, its major metabolite, inhibit xanthine oxidase, thereby limiting the biosynthesis of uric acid and decreasing its serum level and urine excretion and promoting the renal clearance of hypoxanthine and xanthine. These effects suggested its potential usefulness in the therapy of gout; the drug widely used in the treatment of both primary and secondary hyperuricemia. Chronic allopurinol administration for the inhibition of XO is clinically effective against the hyperuricemia associated with gout, but undesirable side effects have prompted efforts to isolate or synthesise other types of XO inhibitors. Moreover, both allopurinol and oxypurinol, owing to their purine-like

structure, are known to undergo conversion to the corresponding nucleotides and may cause interactions with other enzymes involved in the purine metabolism [1].

Chrysin and luteolin with hydroxyl groups at C-5 and C-7 of the flavones skeleton inhibited XO strongly. These results of Nagao et al. 1999 indicated that C-5 and/or C-7 are essential for the inhibition of XO. The present results also show a greater percentage inhibition of XO due to the high content of flavonoids in *Celosia trigyna* extract.

4. Conclusion

Extract of *Celosia trigyna* inhibited XO in a dose dependent manner, the total flavonoid, phenol, vitamin C contents were also high in the extract. Finally the extract also shows chelating properties against Fe²⁺. These activities could be attributed to the combined effect of the flavonoids, phenolic acids and Vitamin C present in the extract of *Celosia trigyna*. Therefore, *Celosia trigyna* could be explored for the prevention and management of hyperuricaemia conditions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest exist.

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