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Nutritional Compositions and *In-vivo* Antioxidant Effect of *Corchorus olitorius* Ethanol Leaf Extract in CCl4-induced Oxidative Stress in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author ANCO designed and supervised the study. Author JN managed and performed the experimental and statistical aspects of the study. Author VHAE guided and supervised the antioxidant aspects of the study. Author UCO wrote the protocol and first draft of the manuscript while author PUE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Oxidative stress has been implicated in the pathophysiology of various disease conditions with concomitant toll on the body's defense mechanism against free radicals. To continuously sustain and support the efficiency of the body's antioxidant defense system, natural plant sources are required. Thus, the need for alternative options especially of plants that are neglected and under-utilized. Hence, this study aimed at investigating the proximate and phytochemical compositions and *in-vivo* antioxidant effect of ethanol leaf extract of *C. olitorius* on antioxidant enzyme activities in CCl_4-induced oxidative stress in Wistar rats.
Methods: Thirty albino rats of Wistar strain (120-150g) were divided into six groups (A – F) of five rats each. Groups A, B and C served as test groups and were administered 200 mg/kg, 400 mg/kg and 600 mg/kg doses of *C. olitorius* leaf extract respectively while Group D served as normal control. Groups E and F served as the positive and negative controls and were administered 50 mg/kg Silymarin and distilled water respectively. The administration lasted for 15 days after which blood was collected via cardiac puncture.

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Results: Findings showed that the leaf was rich in total phenol (21.47 ± 0.00 mgGAE/g) and tannin (23.34 ± 0.75 mgTAE/g) with little quantity of oxalate (0.48 ± 0.09 mg/g), cardiac glycosides (0.30 ± 0.07 %) and phytate (0.25 ± 0.01 %). The result of the proximate composition revealed that the leaf was rich in carbohydrate (44.16 ± 1.21 %), ash (20.31 ± 0.51 %) and protein (11.29 ± 2.06 %) with negligible quantity of lipid (0.46 ± 0.11 %). More so, the activities of superoxide dismutase, catalase and glutathione peroxidase were all increased in the extract treated group when compared to the controls.
Conclusion: From the above findings, it can be concluded that the ethanol leaf extract of *C. olitorius* may possess exploitable nutritional components and potential antioxidant activity against the debilitating effects of free radicals.

Keywords: Corchorus olitorius; carbon tetrachloride; oxidative stress; antioxidant; superoxide dismutase; catalase; glutathione peroxidase.

1. INTRODUCTION

Oxidative stress has been implicated as the leading factor in the pathogenesis of a variety of debilitating diseases such as cancer, autoimmune disorders, arthritis, cardiovascular conditions and in aging [1-3]. This is due to the over-production of reactive oxygen species (ROS) including superoxide anion, hydroxyl radical, anoxide etc. that significantly increase the rate of oxidation of several biological membrane components, organelles, nucleic acids, proteins, and polyunsaturated fatty acids. When the body's antioxidant defense system is overwhelmed by these free radicals, there seems to be a tilt in favor of prooxidation affecting essential biomolecular cell components through lipid peroxidation. One free radical generating species of immense biochemical relevance is carbon tetrachloride (CCl_4). It is used as a chemical feedstock for the industrial manufacture of various products ranging from aerosols to resins and as extraction solvent. Intracellularly, CCl_4 is converted to trichloromethyl ($^{\cdot}CCl_3$) by the action of a specific cytochrome P_{450}, which in turn reacts with oxygen initiating a cascade of deleterious effects [2,4]. These reactions thus interfere with normal metabolic processes [3] causing a host of clinical manifestations associated with liver, kidney, lung and heart diseases.

To continuously sustain and support the body's natural enzyme antioxidant system, fruits and vegetables offer viable natural options hence the need for their scientific evaluations for potential therapeutic efficacies. This is because plants are believed to contain phytochemicals that can actively mop up and neutralize free radicals thereby protecting against CCl_4-induced disease disorders [3].

C. olitorius, also known as *Ahihara* in Igbo, is a leafy vegetable that belongs to the family *Tiliaceae*, and commonly called Jute mallow in English and "*Ewedu*" in the Southwestern Nigeria [5]. It is an annual herb with a slender stem and an important green leafy vegetable in many tropical areas such as Nigeria [6]. In West African countries including Ghana, Nigeria and Sierra Leone, the vegetable is cultivated for the stem, bark, which is used in the production of fiber (Jute), and its mucilaginous leaves, which are also used as food vegetable [7]. The leaves (either fresh or dried) are cooked into a thick viscous soup or added to stew or soup and are rich sources of vitamins and minerals [7]. Traditionally, in some parts of Nigeria, leaves' decoction is used for treating iron deficiency, folic acid deficiency, ascites, pains, piles, tumors, gonorrhea, and fever [8,9].

Upon the foregoing background, the study aimed to investigate the proximate and phytochemical compositions in addition to the *in-vivo* assessment of the effect of the plant leaf extract of *C. olitorius* on antioxidant enzymes in Wistar rats.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh green leaves of *C. olitorius* were purchased from Eke-Awka market in Awka South Local Government Area of Anambra State, Nigeria. The plant sample was identified and authenticated by a taxonomist at the Department of Botany, Nnamdi Azikiwe University, Awka and specimen deposited at the herbarium of the Department. The leaves were washed over running tap water and then in doubly distilled water to remove dirt, and thereafter, was air-dried at room temperature for 14 days. The dried samples were ground with a Corona manual grinder into a fine powder and stored in an airtight container until further use.

2.2 Extraction of Plant Materials

Exactly twenty grams (20g) of the ground sample was soaked in 200 ml of 70 % ethanol and was allowed to stand for 48 hours at room temperature with intermittent stirring. The mixture was filtered through Whatman paper No. 4 with the aid of a vacuum filter and the filtrate was evaporated at 60˚C using a water bath (Techmel and Techmel, 420, USA). The dried residue was weighed and reconstituted in 70 % ethanol at a concentration of 10 mg/ml and stored at 4˚C in a refrigerator until further use.

2.3 Experimental Animals

A total of thirty adult Wistar rats weighing between 150 and 200 g were purchased from Onyewuchi Farms, Ifite, Awka and allowed to acclimatize for a period of seven days in the Animal house of the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka. The rats were kept in standard cages with saw dust as bedding and fed with commercial rat chow and water *ad libitum*. They were handled ethically according to the standards provided by the National Institute of Health on Animal Handling and Safety.

2.3.1 Animal grouping and dose administration

The animals were grouped into six (A - F) of five rats per cohort. Test groups A, B and C were administered 200 mg/kg, 400 mg/kg and 600 mg/kg body weights of plant leaf extract respectively. Group D was the normal control and was administered distilled water. Group E served as the positive control and was administered 50 mg/kg of the standard reference drug, Silymarin through oral gavage while Group F was designated the negative control and was administered distilled water.

2.3.2 Induction of oxidative stress in animals

Carbon tetrachloride (CCl_4) was administered intraperitoneally using olive oil as a vehicle in the ratio of 1:1 (0.5 ml/kg body weight) to induce oxidative stress in accordance with previously established methods [10]. Administration of plant extract as well as standard were done orally for 14 days. Previous study revealed that *C. olitorius* at high doses of 2500, 5000 and 7500 mg/kg body weight had no apparent toxic and lethal effects on the animals, which probably indicate that the extract has high safety index [11].

2.3.3 Blood sample collection

On the fifteenth day, the rats were anaesthetized with chloroform after overnight fasting. Blood was drawn slowly through cardiac puncture, collected into plain bottles, and allowed to clot. Thereafter, the blood samples were centrifuged at 3000 rpm for 10 minutes. The sera obtained were transferred into another vial for enzyme assay.

2.4 Sample Analysis

2.4.1 Proximate analysis of *C. olitorius* leaf extract

The moisture, ash, crude fiber and crude fat were determined according to the methods of Association of Official Analytical Chemists [12]. Crude protein was determined by the Micro-Kjeldahl method as proposed by AOAC [12]. The total percent carbohydrate content was estimated by the difference of 100 of the other proximate components as reported by Yerima and Adam [13] using the following formula:

Total Carbohydrate (%) = 100 - (% Moisture + % Ash + % Crude fibre + % Crude protein + % Fat)

2.4.2 Qualitative and quantitative phytochemical analysis of *C. olitorius* leaf extract

The qualitative phytochemical screening of the plant leaf extract of *C. olitorius* was carried out according to the method of Usunobun et al. [14], while the quantitative phytochemical contents were carried out as reported below: Total phenolic content and flavonoids were determined by modified colorimetric tests as described by Barros *et al* [15]. Phytate content was determined using the method of Young and Greaves [16]. The saponin content of the extract solution was determined by the method of Obadoni and Ochuko [17] while oxalate was determined according to Osagie [18]. Tannin, cardiac glycoside and terpenoids were determined according to method of AOAC [12]. The alkaloid content was determined by the method of Harborne [19]. Sterol was determined by the Libermann-Burchard's test.

2.5 *In-vivo* Antioxidant Enzyme Assays

2.5.1 Assay of superoxide dismutase activity

The *in-vivo* superoxide dismutase (SOD) activity was assayed by the method of Sun and Zigma [20] based on its ability to inhibit the auto-oxidation of epinephrine determined by the

increase in absorbance (480nm). The enzyme activity was calculated by measuring the change in absorbance with UV-VIS spectrophotometer (Axiom 752) at 480 nm for 3 minutes.

2.5.2 Assay of catalase activity

In-vivo catalase activity was determined according to the method as described by Sinha [21]. It was assayed spectrophotometrically (Axiom 752) at 620 *nm* and expressed as micromoles of H_20_2 consumed/min/mg protein at 25^oC.

2.5.3 Assay of glutathione peroxidase (GPx) activity

The method proposed by Rotruck et al. [22] was used to assay for *in-vivo* GPx activity. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer (Axiom 752 UV-VIS). One unit of peroxidase is defined as the change in absorbance/minute at 430 nm.

2.6 Determination of Malondialdehyde Levels

Malondialdehyde (MDA) levels, as an index of lipid peroxidation, reacts with thiobarbituric acid (TBA) to give a complex pink colour. This was used to assess lipid peroxidation using the method of Budge and Aust [23]. Absorbance was measured in an Axiom 752 UV-VIS spectrophotometer at 532nm against the blank. Malondialdehyde level (in µM) was calculated using the molar extinction coefficient for MDA-TBA complex of 1.56 x 105 $M^{-1}cm^{-1}$.

2.7 Data Analysis

Data was analyzed using GraphPad Prism 5 Program (GraphPad Software, San Diego, CA, USA). Descriptive statistics and One-way Analysis of Variance were used to statistically test the data. Tukey HSD *post-hoc* test was used to indicate exact point of significance between means of groups. The results were expressed as Mean ± Standard Deviation and values were considered significant at $p < 0.05$ of 95% confidence interval.

3. RESULTS

3.1 Proximate Analysis

Table 1 shows the proximate analysis of the leaf of *C. olitorius.* Result indicated that the plant is rich in carbohydrate (44.16 ± 0.00 %), ash (20.31±0.51 %) moisture (19.49 ± 0.82 %), with considerable protein content (11.29 ± 2.06 %), fibre (5.26 ± 0.14%) while total lipid (0.46 ± 0.11 %) was present in minute quantity.

Table 1. Proximate analysis of *C. olitorius* leaves

Parameters	Content (%)
Ash	20.31 ± 0.51
Crude Protein	11.29 ± 2.06
Moisture	19.49 ± 0.82
Total Lipids	0.46 ± 0.11
Crude Fiber	5.26 ± 0.14
Total Carbohydrate	44.16 ± 1.21

Values are expressed as mean ± standard deviation of triplicate determinations

3.2 Qualitative and Quantitative Phytochemical Analysis

Table 2a presents the qualitative phytochemical screening of the leaf of *C. olitorius* indicating the presence of flavonoids, phenols, terpenoids, tannins, steroids and cardiac glycoside while saponin, alkaloids and phlobatannin were absent.

Table 2a. Qualitative phytochemical analysis of *C. olitorius* leaves

Phytochemicals	Result
Flavonoid	+
Phenol	+
Saponin	-
Alkaloid	-
Terpenoid	+
Tannin	+
Sterol	+
Cardiac glycoside	+
Phlobatannin	-

+ = Present; - = Negative

Table 2b presents the quantitative phytochemical screening of the leaf extract of *C. olitorius.* The plant extract indicates a considerable quantity of phenol (21.47 ± 0.00 mgGAE/g) and tannin (23.34 ± 0.75 mgTAE/g) while flavonoid (0.01 ± 0.00 mgCE/g), phytate (0.25 ± 0.01 %), oxalate (0.48 ± 0.09 mg/g) and cardiac glycoside (0.30 ± 0.07 %) were present in minute quantities.

3.3 *In-vivo* Antioxidant Enzyme Activities and Mda Levels

Fig. 1 shows the effect of the leaf extract of *C. olitorius* on superoxide dismutase, catalase, glutathione peroxidase and malondialdehyde

levels in Wistar rats. Result indicated that there was a significant increase ($p < 0.05$) in SOD, catalase, glutathione peroxidase activities *in-vivo* and malondialdehyde levels were significantly decreased ($p < 0.05$) in the extract treated group when compared with the standard control.

Table 2b. Quantitative phytochemical analysis of *C. olitorius* leaves

Phytochemicals	Concentration
Total Phenol (mgGAE/g)	21.47 ± 0.00
Flavonoid (mgCE/g)	0.01 ± 0.00
Phytate (%)	0.25 ± 0.01
Oxalate (mg/g)	0.48 ± 0.09
Cardiac Glycoside (%)	0.30 ± 0.07
Tannin (mgTAE/g)	23.34 ± 0.75

Values are expressed as means ± standard deviation of triplicate determinations

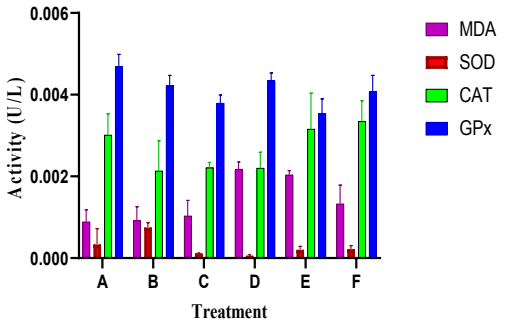

Fig. 1. Effect of *C. olitorius* leaf extract on superoxide dismutase, catalase, glutathione peroxidase enzyme activities and malondialdehyde levels in Wistar rats

4. DISCUSSION

Plants produce different chemical compounds or phytochemical which have been used in a wide range of commercial, medicinal, and industrial applications. The moisture content obtained in this study was found to be 19.49 ± 0.82 %, far lower than 86.35 % reported by Adeniyi et al. [24] for *C. olitorius* leaf and slightly lower than 30.90 % as reported by Onwordi et al. [25] for *C. olitorius* leaf. This result is an indication that the plant can be stored under good conditions without absorbing moisture from the atmosphere leading to microbial contamination [26]. The moisture content of any food is an index of its water activity, and it is used as a measure of storage stability and susceptibility to microbial contamination. Hence, the lower the moisture contents of a food, the higher the storage stability. The ash value observed in this study is consistent with the findings of Onwordi *et al* (25] who reported 21.20 ± 0.80 % of ash content for *C. olitorius* leaf. Ash content reflects the organic mineral matter present in a food sample. The protein content value of the present study agrees with that reported for *C. olitorius* by Onwordi et al. [25]. It has long been established that the performance of foods in biological system depends on the quantity and quality of their proteins [27]. Proteins are major constituents of most structural and cellular components in any living organism as they are composed of amino acids and hence help in cellular growth. *C. olitorius* leaves are poor source of lipid as indicated by the present study. The total lipid contents of 0.46 ± 0.11 % is consistent with 0.32 % for *C. olitorius* leaves as reported by Onwordi et al. [25] but considerably lower to 6.10 % reported for ethanol leaf extract of *C. olitorius* by [28]. Vegetables are poor sources of fat hence good for obese people. The carbohydrate content of *C. olitorius* (44.16 ± 1.21 %) is higher than 31.34 % reported by Onwordi et al. [25] in *C. olitorius* leaves but consistent with the 42.99 % carbohydrate content of ethanol leaf extract of *C. olitorius* reported by Adeniyi et al. [24]. The carbohydrate content obtained for this plant is sufficient to classify *C. olitorius* as a carbohydrate-rich food and hence could supply most of the body's energy requirements.

C. olitorius leaves have been shown to contain numerous phytochemical constituents. These phytochemicals are known to possess therapeutic efficacies which justify their uses in traditional medicine [29]. Sharmila et al. [30] documented that these phytochemicals may be responsible for several pharmacological activities like wound healing, cholesterol lowering and antidiabetic activity. It has long been documented that plant steroids, flavonoids and phenols are antioxidants [31]. The phenol content obtained in this study was to be similar to the findings of Roy et al. [32]. Phenols are a class of aromatic organic compounds with the molecular formula C_6H_5OH and are white, volatile crystalline solids. Similarly, the tannin content of *C. olitorius* obtained in this study was consistent with the findings of Roy et al. [32]. Tannins are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids [33]. Other phytochemicals present in the leaf extract of *C. olitorius* included cardiac glycosides, oxalate, phytate as well as flavonoids. Flavonoids are a group of plant metabolites thought to provide health benefits

through cell signaling pathways and antioxidant effects. These molecules are found in a variety of fruits and vegetables. Flavonoids are polyphenolic molecules containing 15 carbon atoms and are soluble in water. They are known generally to be responsible for colour, taste, prevention of fat oxidation, and protection of vitamins and enzymes [34].

In this study, acute CCl_4 exposure significantly elevated the malondialdehyde (MDA) levels indicating enhanced peroxidation and breakdown of the antioxidant defense mechanisms. Decomposition products of lipid hydroperoxide such as malonaldehyde can cause chaotic cross-linkage with proteins and nucleic acids, which plays an important role in the process of carcinogenesis. In this investigation, administration of ethanol leaf extract of *C. olitorius* at different doses of 200, 400 and 600 mg/kg body weight significantly decreased the MDA levels, suggesting its protective effects against CCl_4 induced oxidative damage. These findings agree with the studies of Airaodion et al. [35] who reported both the ameliorative efficacy of phytochemical contents of *C. olitorius* leaves against acute ethanol-induced oxidative stress in Wistar rats and hepatoprotective effect of *Parkia biglobosa* on acute ethanol-induced oxidative stress in Wistar rats.

All organisms have their own cellular antioxidant defense system composed of both enzymatic and non-enzymatic components. Enzymatic antioxidant pathway consists of SOD, CAT and GPx. Superoxide anion is dismutated by SOD to H_2O_2, which is reduced to water and molecular oxygen by CAT or is neutralized by GPx, which catalyzes the reduction of H_2O_2 to water and organic peroxide to alcohols using GSH as a source of reducing equivalent. Superoxide dismutase (SOD) plays an important role in reducing the effect of free radicals' attack, and SOD is the only enzymatic system quenching molecular oxygen (O_2) to H_2O_2 and plays a significant role against oxidative stress [36]. These radicals have been reported to be deleterious to polyunsaturated fatty acids and proteins [37]. In this study, significant difference was observed in the activity of SOD in *C. olitorius* treated group compared with the control groups. This might be that CCl_4-induced oxidative stress elevated ROS in the liver which SOD tend to combat thereby increasing its activity. *C. olitorius* was able to reduce the ROS generation with subsequent increase in SOD activity due to its high phytochemical content as reported by Orieke *et al* [36].

Catalase contributes to ethanol oxidation by oxidizing a small amount of ethanol in the presence of H_2O_2 generating system to form acetaldehyde [38]. In this study, a significant increase was observed in the activity of catalase in *C. olitorius* treated animals when compared with control. This contradicts the findings of Airaodion et al. [35] who reported a non-significant difference when animals were treated with *Parkia biglobosa*. The activity of catalase in animals treated with *C. olitorius* after the induction of oxidative stress with CCl_4 was significantly increased when compared with those without *C. olitorius* treatment. This might be that CCl_4-induced toxicity generated elevated ROS in the liver which catalase tend to combat, thereby increasing its activity. *C. olitorius* was able to reduce the ROS generation with subsequent decrease in catalase activity due to its high phytochemical content as reported by Orieke et al. [36]. Increased catalase activity in this study following exposure to CCl_4 suggests elevated oxidation. This agrees with the study of Airaodion et al. [35] and Oyenihi et al. [39] who reported a significantly higher CAT activity after ethanol-induced oxidative stress.

Glutathione peroxidase (GPx) is another enzymic antioxidant that acts as a defense against oxidative stress. It directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism. It is known to detoxify hydrogen peroxide and lipid peroxide by donating electron to hydrogen peroxide to reduce it to water and oxygen, thereby protecting macromolecules such as lipids from oxidation. The findings correlate with the works of Ganie *et al* [2] and Shah *et al* [3] who reported significant decrease in the activity of GPx on CCl_4-induced oxidative stress but was restored on administration of *P. hexandrum and N. biserrata* ethanol leaf extracts respectively, suggesting the role of bioactive antioxidant compounds in the plants.

5. CONCLUSION

The plant leaf extract showed considerable proximate and phytochemical contents as well as exhibited appreciable *in-vivo* antioxidant activities. From the above findings, *C. olitorius* appears to have excellent health and medicinal benefits which deserve to be further explored. Going forward, it is advocated that the phytochemical compounds be characterized for further antioxidant studies.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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