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Antioxidant Properties of *Crinum ornatum* Bulb Extract

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Abstract: The antioxidant properties of the methanolic extract of the bulb of *Crinum ornatum* (Ait) Bury of the family Amaryllidaceae was investigated using the UV/Visible spectrophotometer. The extract showed marked activity as a radical scavenger in the experiment using 2, 2–diphenyl–1–picrylhydrazyl radical (DPPH), indicating that the extract of *C. ornatum* has ability to donate hydrogen. The absorbtion is stoichiometric with respect to the number of electron taken up. Concentration of 0.1mg/ml exhibited 67.93% scavenging activity. The antioxidative activity of this compound towards hydrogen peroxide is also reported. A 92.7% inhibition of hydrogen peroxide was observed when compared with control experiment at a concentration of 0.1mg/ml. The extract also showed marked inhibitory effect on peroxydation of tissue lipids in the malonaldehyde (MDA) test. Administration of the crude extract decreased the concentration of thiobarbituric acid reactive substances of rat liver homogenate hence provides a beneficial effect on the brain. At the highest concentration of 0.1mg/ml, 85.5% inhibition of malonaldehyde was observed. This extract may be a candidate for treating pathologies relating to free radical oxidation due to its overall antioxidant effect in scavenging free – radicals and active oxygen species.

Key words: Radical scavengers · Absorbance · 2, 2-diphenyl-1-picrylhydrazyl (DPPH) · Malonaldehyde · Hydrogen peroxide · Lipid peroxidation

INTRODUCTION

Crinum ornatum (Ait) Bury of the family Amaryllidaceae is a bulbous plant with thick wide spreading rich green, glaucous leaves reaching 75cm long by 6cm wide and scape 60 - 100cm high bearing 4-6 white flowers with a broad purple band. It grows well in damp site. This plant is given attention because of its use as an anticonvulsant and its other folk uses in traditional medicine in Nigeria and some other countries in Sub Saharan Africa.

The *Crinum species* are known to be biologically active. The antibacterial and antifungal activities [1], antitumor, immunostimulating and insecticidal activities [2], amongst others have been reported for certain species in this family. Alkaloids have been isolated and their bioactivity investigated. The antioxidant properties has however not been investigated. *C. ornatum* is used as a pilot study in this regard. Free radical pathology is as a result of uncontrolled abnormal radical reactions occurring in cells. Free radical pathology is based on the fact that key biomolecules within cell membranes and in other macromolecular aggregates like nucleic acids are highly susceptible to radical reactions. The idea that the methanolic extract of *C. ornatum* (MECO) contains some antioxidant is a precursor in carrying out these tests. Anti-oxidants are able to prevent the formation of reactive species as free radicals are now recognized to participate in the pathogenesis of a growing number of disorders such as inflammation, asthma, arthritis, neurodegeneration, convulsion, Parkinson's disease, arteriosclerosis and cancer. These reactive oxygen species are known to cause oxidative damage thus altering the structure and function of cells of biological macro-molecules [3]. On the basis of this, natural anti-oxidant are been given much attention in recent times. In this paper, we report the antioxidant properties of methanolic extract of *Crinum ornatum* bulb. This goal was achieved by subjecting the methanolic extract of this plant (MECO) to *in-vitro* antioxidant assay which include the determination of the effect on DPPH radical (2,2-diphenyl-1-picrylhydrazyl). DPPH radical gives strong absorption at 517nm (deep violet color) in visible spectroscopy. The absorption vanishes or is decolorized as the electron becomes paired off in the presence of a free radical scavenger). Other *in-vitro* antioxidant assay

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employed are scavenging effect on hydrogen peroxide (H_2O_2) and inhibition of lipid peroxidation as determined by the thiobarbituric assay on rat liver microsomal preparations.

MATERIALS AND METHODS

Reagents: Thiobarbituric acid (TBA), butylated hydroxyanisole (BHA) and bovine serum albumin (BSA) were obtained from Sigma Chemical Co (St Louis, MO). 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was obtained from Aldrich-Chimie (France) while hydrogen peroxide was obtained from E-Merck (Germany).

Animals: Adult Wistar rats (males) weighing 160 - 220 g (14 weeks old) obtained from the animal House of the Department of Physiology, University of Ibadan were used. The rats were fed with balanced livestock feed from Pfizer, plc and water was also given *ad libitum*.

Plant Materials: Fresh bulbs of *C. ornatum* were obtained in Ibadan, Nigeria and identified and authenticated at the Forestry Research Institute of Nigeria, Ibadan (FHI No 105367). The bulbs were air-dried and ground into a fine powder with a Hammer Mill (Ashai 7500) and kept in non-absorptive nylon for subsequent use.

Extraction Procedure: The powdered bulb (1kg) was extracted with methanol by cold extraction. The mixture was filtered to remove the marc. The combined filterates were evaporated to dryness in a rotary evaporator at 37°C and stored in a desiccator prior to further analysis. Thin Layer Chromatography was employed using silica gel 60 F_{254} precoated plates and solvent system: Ethylacetate/methanol (8:2) to detect antioxidant activity by using DPPH as a spray reagent. Yellow coloration on the spots on the TLC plates indicates that the methanolic extract of *C. ornatum* (MECO) has antioxidant activity after which it was subjected to the following spectrophotometric experiments using UV-Visible spectrophotometer (HE λ 10S Perkin-elmer α RIOOA Recorder, Switzerland).

Free Radical Scavenging Activities:

Extinction of DPPH: 3.94mg of DPPH, a stable radical was dissolved in methanol (100ml) to give 100μM solution. To 3.0ml of methanolic solution of DPPH was added 0.5ml of MECO in methanol (from stock solution 0.1g in 100ml). The decrease in absorption at 517nm of DPPH was measured after 10min. The actual decrease in absorbtion induced by the test compound was calculated by subtracting that of the control. The percentage inhibition was also calculated. All tests and analyses were run in triplicates and averaged [4].

Scavenging Effects on Hydrogen Peroxide: Spectrophotometric determination of MECO to scavenge hydrogen peroxide was carried out at 285nm using the method of Beers and Sizer [5]. A solution of 2mM hydrogen peroxide was prepared in phosphate-buffered (PBS) saline pH 7.4. Solution of MECO at the following concentrations, 0.005mg/ml, 0.01mg/ml, 0.015mg/ml, 0.025mg/ml, 0.05mg/ml and 0.1mg/ml was added to hydrogen peroxide solution. Absorbance of the hydrogen peroxide at 285nm was determined spectrophotometrically after 10min against a blank solution containing MECO in phosphate- buffered saline without hydrogen peroxide. All tests and analyses were run in triplicates and averaged.

Antioxidant Activity:

Inhibition of Lipid Peroxidation: The formation of malonaldehyde (MDA) was used as a measure of lipid peroxidation and was determined by the thiobarbituric assay on rat liver microsomal preparations [6] using UV- Absorbance Spectroscopy.

Microsomal Preparation: The livers of male Wistar rats (2) were removed by cervical dislocation. The livers were washed with cold 1.15% KCl solution and then weighed. These were homogenized in 3 volumes of homogenizing buffer. Homogenizing buffer is a mixture of 20ml, 50mM Tris - HCl and 50ml 1.15% KCl. The mixture was made up to volume with 1L distilled water. The pH of the resulting solution was adjusted to 7.4 with 1M HCl. The homogenate obtained was centrifuged at 9000g for 20min at 4°C to obtain the supernatant. The supernatant was then centrifuged at 105,000g for 60min at 4°C to obtain the microsomal fraction. This was then suspended in 0.25M sucrose. The protein concentration of the microsomal fraction to be used for lipid peroxidation assay was determined by Biuret method [7].

Lipid Peroxidation Assay: The reaction mixture composed of 0.1ml microsomal suspension (0.85mg of protein/ml), methanolic extract (MECO) with concentrations ranging from 0.01, 0.005, 0.01, 0.015, 0.025, 0.05 and 0.1mg/ml. 0.2ml of an aqueous solution of $FeSO_4$ ($5x10^{-3}$M), 0.2ml of $2x10^{-3}$ M H_20_2 and 0.2ml of $5x10^{-3}$M ascorbate were

added to the resulting mixture. The mixture was incubated at 37°C for 6hrs in capped tubes. After a cooling period of 10min in ice, 0.5ml of 10% aqueous solution of trichloroacetic acid and 0.5ml of 0.75% solution of thiobarbituric acid were added. The solution was boiled at 95°C for 20min and then centrifuged for 10min at 2500rpm. The supernatant was transferred into acid resistant tubes and centrifuged at 16000rpm for 10min. The absorbance of the resulting clear solution was determined at 532nm with phosphate buffer as blank [7].

Statistical Analysis: Data (Absorbance measurements) are expressed as mean absorbance ± S.D. of triplicate analysis. Statistical analysis was performed by a one-way analysis of variance (ANOVA) for more than two means while Student's t - test was used for comparison between two means. Values of $P \leq 0.05$ were taken to be statistically significant.

RESULTS AND DISCUSSION

Free Radical Scavenging Activity

Extinction of DPPH: The free radical scavenging activity was evaluated by the decrease in absorbtion of the stable radical 2,2-diphenylpicryl hydrazyl radical (DPPH) at 517nm. MECO decolorized DPPH due to its hydrogen donating ability [8]. The absorption is stoichiometric with respect to the number of electron taken up. Scavenging activity of MECO on DPPH is shown in Table 1. Concentration of 0.1mg/ml exhibited 67.93% scavenging activity. However Butylated hydroxylanisole (BHA), a known antioxidant at this concentration exhibited marked scavenging activity (>90%).

Scavenging Effects of Methanolic Extracts of C. Ornatum on Hydrogen Peroxide: The extract was capable of scavenging hydrogen peroxide in a concentration dependent manner after 10min of incubation at 285nm. A 92.7% inhibition of hydrogen peroxide was observed when compared with control experiment at a concentration of 0.1mg/ml. The scavenging activity of MECO on hydrogen peroxide is shown in Table 2. It has been shown that H_2O_2 has only a weak activity to initiate lipid peroxidation, but its activity as an active-oxygen specie comes from its potential to produce the highly reactive hydroxyl radical through the Fenton reaction [9]. Removal of OH radical is one of the most important effective defence of a living body against diseases, therefore any compound with antioxidant activity capable of doing this might contribute towards the total and partial alleviation of this damage [10]. Therefore, the ability of the extract to scavenge H_2O_2 contributes to inhibition of the peroxidation of lipid.

Thus the result of this study (Table 2) shows that *C. ornatum* possesses antioxidant activity which could exert a beneficial action against pathological alterations caused by the presence of hydroxyl radical.

Effect of Methanolic Extract on Lipid Peroxidation: Administration of the crude extract decreased the concentration of thiobarbituric acid reactive substances of rat liver homogenate hence provides a beneficial effect on the brain. Table 3 shows that at the highest concentration of 0.1mg/ml, 85.5% inhibition of malonaldehyde was observed. The activity was dose dependent.

Table 1: A_{517} Decrease and Inhibition (%)

Compound	Concentration (mg/ml)	Absorbance at 517nm**	Inhibition %
Control	0.1	1.484 ± 0.0000	0
MECO	0.1	0.476 ± 0.0007	67.93
BHA	0.1	0.018 ± 0.0017	98.79

*The reduction in A_{517} of DPPH (100µM) caused by the methanolic extract and Butylated hydroxylanisole was measured in triplicate after 10min
**Each value represents the mean ± standard deviation of triplicate analysis
$P \leq 0.05$ compared with control Student's t - test

Table 2: Effects of methanolic extract of *C. ornatum* on H_2O_2*

Compound	Concentration (mg/ml)	Absorbance at 285nm**	Inhibition %
Control	0.1	1.979 ± 0.0000	0.00
MECO	0.005	1.435 ± 0.0006	27.49
MECO	0.01	0.982 ± 0.0012	50.38
MECO	0.015	0.599 ± 0.0006	69.73
MECO	0.025	0.341 ± 0.0006	82.77
MECO	0.05	0.145 ± 0.0006	92.67
MECO	0.1	0.143 ± 0.0006	92.77

*The scavenging activity of methanolic extract on hydrogen peroxide was measured at 285nm
**Each value represents the mean ± standard deviation of triplicate analysis. P= 0.05 compared with control Student's t - test

Table 3: Concentration effect of *C. ornatum* on MDA Production*

Compound	Concentration (mg/ml)	Absorbance at 532nm**	Inhibition %
Control	0.1	0.099 ± 0.0000	0.00
MECO	0.005	0.096 ± 0.0000	3.03
MECO	0.01	0.088 ± 0.0008	11.10
MECO	0.015	0.075 ± 0.0000	24.23
MECO	0.025	0.066 ± 0.0009	33.34
MECO	0.05	0.058 ± 0.0000	41.41
MECO	0.1	0.014 ± 0.0006	85.50

*The reaction mixture composed of (i) microsomal suspension (ii) a solution of methanolic extract with doses ranging from 0.005, 0.01, 0.015, 0.025, 0.05 and 0.1mg/ml and (iii) aqueous solution of $FeSO_4$ ($5x10^{-3}$M). Detection of the malonaldehyde – thioabarbituric adduct was recorded at 532nm
**The results represent the mean ± standard deviation of triplicate analysis. P = 0.05 compared with control Student's t - test

The results as presented in Table 3 demonstrated that MECO dramatically prevented microsomal preparation from MDA formation which substantiate its radical scavenging activity and which is also in good agreement with the previous assays. It has been shown that the brain contains low level of vitamin E and selenium dependent glutathione peroxidase activity and hence the brain is more prone to lipid peroxidation [11]. Therefore with the crude extract, anti-lipid peroxidation, anti-superoxide formation and hydroxyl radical scavenging activity were demonstrated *in vitro*.

CONCLUSION

This study has provided evidence that the methanolic extract of *C. ornatum* has antioxidant activity. It has capacity to scavenge free radicals as observed in the extinction of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The absorbtion is stoichiometric with respect to the number of electron taken up. Concentration of 0.1mg/ml exhibited 67.93% scavenging activity, indicating that the extract of *C. ornatum* has ability to donate hydrogen. The antioxidative activity of this compound towards hydrogen peroxide is also reported. A 92.7% inhibition of hydrogen peroxide was observed when compared with control experiment at a concentration of 0.1mg/ml. The extract also showed marked inhibitory effect on peroxydation of tissue lipids in the MDA test. Administration of the crude extract decreased the concentration of thiobarbituric acid reactive substances of rat liver homogenate hence provides a beneficial effect on the brain. At the highest concentration of 0.1mg/ml, 85.5% inhibition of malonaldehyde was observed. This extract may be a candidate for treating pathologies relating to free radical oxidation due to its overall antioxidant effect in scavenging free - radical and active oxygen species. These results indicate that the extract is a free radical inhibitor, a primary antioxidant that reacts with free radicals. There was significant decrease as the concentration of the extract increases as demonstrated in its scavenging activity with hydrogen peroxide. This is made possible by its activity as an active oxygen specie. And inhibitory effect on the peroxidation of tissue lipids in the MDA test are all positive tests indicating the potential of the methanolic extract of *C. ornatum* as an antioxidant. These effects may therefore prove useful in the treatment of pathologies in which free radical oxidation plays a major role. This research has therefore proved to us that the *Crinum Species* are potential antioxidants.

RECOMMENDATION

The importance and uses of herbs cannot be over emphasized because local/indigenous people especially in developing nations rely so much on their use. Research and findings have also shown that plants will continue to provide leads in drug production. This plant *Crinum ornatum* showed promising results as an antioxidant specie which is why more *in-vitro* and *in-vivo* assays needs to be carried out to isolate and characterize the active chemical compounds in this plant.

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