Trop J Nat Prod Res, April 2019; 3(4):145-148

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

# Extract of *Costus spectabilis* Attenuates $H_2O_2$-Induced Cataract in Cultured Rat Lenses

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ARTICLE INFO

*Article history:*
Received 08 April 2019
Revised 04 May 2019
Accepted 06 May 2019
Published online 07 May 2019



ABSTRACT

Ethno-medicinally, fresh sap from the rhizome of *Costus spectabilis* (CS) or its water extract has been used as eye drop in the management of cataract. The present study is aimed at verifying this claim using $H_2O_2$-induced cataract model on isolated, cultured rat lenses. The phytochemical constituents of the extract were verified according to standard methods. Isolated lenses from adult Sprague-Dawley albino rats were cultured in 24-well plates and treated with or without 0.5 mM of $H_2O_2$, 0.5 mM of $H_2O_2$ plus 0.5 mg/mL, 1 mg/mL, or 2 mg/mL of CS for 24 hrs. Cataract or lens opacity was assessed by scoring and measurement of pixel intensity. Total protein (TPC), Reduced glutathione (GSH), Superoxide Dismutase (SOD) and Malondialdehyde (MDA) level or activity in lens homogenate was measured. Hydrogen peroxide 0.5 mM induced obvious opacity of the lens, but 0.5 mg/mL of CS significantly ($P \leq 0.001$) decreased it. Other doses of CS demonstrated mild decrease in cataract formation or opacity of lens. Furthermore, 0.5 mM of $H_2O_2$ caused significant decrease ($P \leq 0.05$) of TPC, GSH and increase of MDA level in the lens, but this has not been significantly reversed by CS (0.5-2 mg/ml). The identified phytochemical constituents of the extract include; Steroids, Triterpenes, saponins and flavonoids. The observed activity of the extract may be attributed to the presence of one or some of these constituents.

***Keywords:*** Cataract, Opacity of lens, Pixel intensity, Phytochemical constituent

## Introduction

Cataract is the leading cause of blindness worldwide accounting for 50% of all cases.[1] The prevalence of cataract in the tropics rises more due to extreme temperatures and exposure to UV-light.[2] A 5th of adult 40 years and above in Nigeria is reported to have some degree of lens opacity [3]. Data from The ophthalmological Society of Nigeria (2005) indicates that about 1.2 million people in Nigeria are blind due to cataract and about 4.08 million people have low vision. The report also estimated that the number of blind and low-vision people would almost double by the year 2020 unless concerted action is taken.[4]

Currently, the only clinically accepted approach for the treatment of cataract is surgery [5]. The cost of the surgery hinders many individuals access to treatment in different parts of the world and has increased the need for less expensive, non surgical approach to cataract treatment [6]. Thus, a pharmaceutical intervention, a non surgical approach, that is likely to be cheaper and effective are intensively sought after.

In an attempt to provide alternative approach toward treatment of cataract, several plant species were investigated. Plant extracts, such as extract of *Ocinum sanctum*, aqueous garlic extract, onion juice, as well as fraction of flavonoids extracted from *Emilia sonchifolia* and polyphenolic compounds of *Camelia sinensis* have been proven toameliorate selenite-induced cataract formation by enhancing antioxidant enzyme activity, inhibiting free radical formation and lipid peroxidation.[5]

Previous report revealed that fresh sap from the rhizome of *Costus spectabilis* or the water extract has been used as eye drop in traditional medicine for the treatment of cataract.[7] In this work, an attempt was made to validate this folkloric claim and identify the phytochemical constituents of the extract.

## Materials and Methods

*Identification, Collection and Preparation of the Plant Material*

The whole plant of *Costus spectabilis* was collected from Shika, Zaria, Kaduna State on September, 2018. A sample from the freshly collected plant material was identified by a taxonomist, Mal. U.S Gallah of Bioresources Unit, National Research Institute for Chemical Technology (NARICT) Basawa Zaria with a voucher No. 1611. The rhizome of the plant material was then cut, peeled off to remove the brown papery scales, sliced and dried under the shade. The dried plant material pulverized, weight and packed in a plastic container.

*Extraction of the Plant Material*

The powdered plant material (470g) contained in a glass jar was extracted by maceration in 5L of aqueous ethanol (70%$^{v}/_{v}$) at room temperature for 72hrs. The content of the jar was then filtered through a cotton plug and subsequently through a filter paper (Whatman no.1). Finally, the filtrate was concentrated and dried at low temperature on rotary evaporator given a weight of 40g of the extract.

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Citation: Shehu S, Shehu UF, Shehu S, Danmalam UH, Danjuma NM. Extract of *Costus spectabilis* Attenuates $H_2O_2$-Induced Cataract in Cultured Rat Lenses. Trop J Nat Prod Res. 2019; 3(4):145-148. doi.org/10.26538/tjnpr/v3i4.7

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

*Preliminary Phytochemical Screening*
The aqueous ethanol extract was screened for the presence of different classes of phytochemical compounds using standard method of analysis described by Evans [8].

*Anticataract Studies*
*Animals*
Thirty Wistar rats of either sex weighing 150-200g were used for the study. The rats were obtained from the animal house stock of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria and were handled in accordance with the guidelines as per the ABU Zaria Animal Ethical Committee. The study commenced after getting approval by the university Animal Use and Care Committee with approval number ABUCAUC/2017/006.

*Lens Collection and Culture*
Rats were anesthetized with chloroform and then sacrificed. The eye balls removed and with the aid of surgical blade, lenses isolated through a posterior approach. The Freshly dissected lens was then rolled on filter paper to remove any adherent vitreous. Each isolated lens then placed in a Falcon plastic culture plate (24-well) containing 2 mL of artificial aqueous humor (NaCl 140 mM, KCl 5 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, NaH $(PO4)_2$ 0.5 mM, $CaCl_2$ 0.4 mM), also added into the medium are 100 µg/ml streptomycin, and 100 IU/mL penicillin to prevent bacterial contamination and the pH of the culture maintained at 7.8 as specified by Lokesh.[9] At room temperature, the lenses were then incubated for 2 hours. Damaged lenses that developed artifactual opacities discarded and only transparent ones were selected and used for the experiment.

*Hydrogen peroxide-induced cataract*
The procedure for cataract induction was conducted as described by Suchita *et al*.[10] Hydrogen peroxide was added to the culture medium (artificial aqueous humor) to give a final concentration of 0.5 mM. Transparent lenses were then divided equally into five different groups, each group comprising of six lenses (6) to serve as normal, control, and three test groups. The lenses in the normal group were cultured in artificial aqueous humor alone. Lenses in the control group were cultured in artificial aqueous humor plus 0.5 mM hydrogen peroxide, while those in the test group were cultured in artificial aqueous humor with 0.5 mM hydrogen peroxide plus 0.5mg/ml, 1mg/ml or 2 mg/mL concentrations of the extract. The Lenses were then incubated for 24 hours at room temperature. Finally, the lenses were examined for opacity, washed, weighted and processed for the estimation of biochemical parameters.

*Visual Assessment and Image Analysis*
The procedure as described by Shruthi *et al*.[11] was used. Lens appearance was assessed and scored before and after treatment at each time point by visual inspection. The scoring system is based on the method of Dickerson *et al*.,[12] where 0 = clear lens, 0.2 = spots, 0.5 = cloudy, and 1 = opaque. For imaging, lenses were placed in a petri dishes containing medium and the image captured with an Olympus DP20 camera, which uses a 1200 x 1600 pixel Bayer mask charge-coupled device. The images then converted in 16-bit grayscale images and analyzed with Image j software to obtain the mean pixel intensity of the image of each lens within a group. This will serve as a measure of opacity of the lens (where an increase in the intensity value correspond to increase opacity of the lens).[11]

*Biochemical Estimation*
*Estimation of Total Protein (TPC)*
The protein content of the lenses were determined by the method of Lowry *et al*.,[13] using bovine serum albumin as the standard. It was evaluated as mg/g of fresh weight of the lens. 2 mL of alkaline copper sulphate reagent was added to 0.2 mL solution of lens homogenate solution after centrifugation of lens homogenate at 10,000 rpm and allowed to stand for 10 min at room temperature. 0.2 mL of Folin Ciocalteau solution was then added, and the tube shaken to mix the solution. After 30 min, the absorbance was measured at 660 nm.

*Reduced glutathione (GSH)*
Reduced glutathione (GSH) concentration measurement was done according to Ellman[14] as describe by Rajagopalan *et al*.[15] To 150 µL of lens homogenate (in phosphate - saline buffer pH 7.4), 1.5 mL of 10% trichloroacetic acid was added and centrifuge at 1500xg for 5 minute. 1 mL of the supernatant was treated with 0.5 mL of Ellman's reagent (19.8 mg of 5,5-dithiobis nitro benzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate) and 3 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. The quantity of GSH was obtained from the graph of the GSH standard curve.

*Malondialdehyde (MDA)*
Lipid peroxidation as evidenced by the level of malondialdehyde (MDA) formation was measured by the method described by Akanji *et al*.[16] Lens homogenate (150µl) was treated with 2ml of TBA-TCA-HCL (15% Trichloroacetic Acid (TCA) solution, 0.37% Thiobarbituric Acid (TBA) solution, 0.25 N HCL) reagent (1:1:1 ratio) and placed in a water bath at 90°C for 60 minute , the mixture was cooled and centrifuged at 3000rpm for 5 minute and the absorbance of the pink supernatant (TBA-Malonaldehyde complex was then measured at 535 nm. Malonaldehyde formed was then calculated using the Molar extinction coefficient of $1.56x\ 10^{-5}\ cm^{-1}\ M^{-1}$.

TBARS Conc. (nmol/mg protein) = Absorbance of sample / $1.56x10^{-5}$ x protein Conc. (mg).

*Superoxide Dismutase Estimation*
Superoxide dismutase (SOD) was evaluated as described by Fridovich [17]. Lens homogenate of 0.1 mL was diluted in 0.9 mL of distilled water to make 1:10 dilution of micro some. An aliquot mixture of 0.2 mL of the diluted microsome was added to 2.5 mL of 0.05 M carbonate buffer. The reaction was started with the addition of 0.3 mL of 0.3 mM Adrenaline. The reference mixture contained 2.5 mL of 0.05 M carbonate buffer, 0.3 mL of 0.3 mM Adrenaline and 0.2 mL of distilled water. The Absorbance was measured over 30 seconds up to 150 seconds at 480 nm.

Increase in absorbance per minute = change in absorbance/2.5

% Inhibition =100 - {(Increase in absorbance for sample/Increase in absorbance of blank) x 100}

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of Adrenaline to adenochrome in 1 minute.

*Statistical Analysis*
After six determinations of each of the parameters, the results were expressed as Mean ± Standard Error of Mean (SEM) and the data were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's Post hoc test. The differences between means were considered significant at $P \leq 0.05$.

## Results and Discussion

At the end of the 24 hr treatment, lenses in the untreated peroxide group exhibited an obvious loss in transparency higher than lenses in the extract treated and normal control groups (Figure 1). The Result in Figure 2 shows the degree of lens opacity measured as grey image pixel intensity. The result indicates that incorporation of $H_2O_2$ during 24 hr in the culture media significantly ($P \leq 0.001$) increased lens opacity or cataract formation when compared with the normal control. When the extract at the three tested doses (0.5, 1 and 2 mg/mL) was applied simultaneously with the $H_2O_2$, the increase in opacity is decreased. A Significant reduction ($P \leq 0.001$) was observed at 0.5mg/ml of the extract. The findings can be supported by the result in Figure 3, which indicates the score of opacity of lenses in the different groups. A significant reduction ($P \leq 0.05$) in the score of opacity was seen at 1 mg/mL and 0.5 mg/mL doses of the extract.
Data from this study thus suggests the efficacy of the extract in reducing opacity and possibly restoring the lens bio-molecules and consequently

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

prevention of cataract induction at a lower dose (0.5 mg/mL) but not at the higher doses. Though the reason for the significant reduction in severity of cataract by the extract at lower dose when compared with the higher doses is not yet understood, the result is comparable to a study reported by Yun Li *et al*.,[18] where the efficacy of alpha lipoic acid on $H_2O_2$-induced cataract was investigated. Their findings showed that 1mM of alpha lipoic acid used in their study demonstrated to be most effective compared to 2 mM alpha lipoic acid a higher dose or 0.5 mM alpha lipoic acid the lowest dose used. The result of the present study is also in conformity with what was reported by Ajani *et al*.,[19] where chemo-preventive and remediation effect of *Hydrocotyl bonariensis* leave extract in galactose-induced cataract was investigated. The result of their study indicated that the extract demonstrated efficacy in reducing aggregation of lens protein and consequently prevention of cataract induction at a lower dose (500 $mgkg^{-1}$) but not at a higher dose of 1000 $mgkg^{-1}$.

The result of the GSH status indicates that lens placed on 0.5 mM $H_2O_2$ had a significant ($P \leq 0.05$) decrease of reduced glutathione (GSH) level when compared with the normal control (Tab. 1). When CS was added at the three different doses, the level of GSH improves but not significantly different from the untreated (toxic) group. As presented in Table 1, there is a decrease in SOD level in the peroxide group compared to the treated and normal group, but the difference is also not significant. Also, the evaluation of total protein content of the lens in various groups indicates that 0.5 mM $H_2O_2$ caused a significant ($P \leq 0.05$) reduction in lens total protein concentration compared to the control. But the result shows no significant restoration of the protein levels when the different concentrations of the extract were simultaneously administered. It can be stated therefore, that, although the extract has demonstrated some degree of cataract amelioration, it has not significantly restored the level of these bio-molecules. The findings of our study can be supported by the report of Ajani *et al*.,[19] where they reported that administration of 1000 $mgkg^{-1}$ of *Hydrocotyl bonariensis* leave extract in galactose-induced cataract reduced cataract index and lens peroxidation, but did not increase the lens antioxidant status significantly.

Data obtained from the study shows that addition of 0.5 mM $H_2O_2$ caused significant increase ($P \leq 0.05$) in lens peroxidation (Table 1) as the malondialdehyde (MDA) observed for lens of rats placed on $H_2O_2$ was significantly higher than that obtained for the normal control. Incorporation of any of the three doses of CS reduces the extent of lipid peroxidation but not to a significant level.

Reactive oxygen species are responsible for protein oxidation which decreases the final protein concentration of tissues during pathophysiological conditions.[20] Previous studies have shown the strong connection between $H_2O_2$ overproduction and cataract development. Cataract patients had elevated $H_2O_2$ in both the aqueous body and lens ranging from 7 to 30-fold higher than normal.[21] Lens organ *ex vivo* culture with $H_2O_2$ in the medium is a common experimental model of cataract. This type of cataract is characterized by loss of GSH, SOD, proteolysis due to oxidation and increased levels of lipid peroxidation in the lens tissue. The present study showed significant decrease in GSH, total protein and an increased lipid peroxidation shown by malondiadehyde (MDA) level as presented in Table 1. Therefore, the study is in accordance with previous findings. It is important to note that, though the extract of CS has not significantly restored the level of these bio-molecules, their levels were maintained relatively closer to the normal control group (Table 1).


**Figure 1:** Images of the representative lens in each treatment/control groups taken after 24 h of treatment

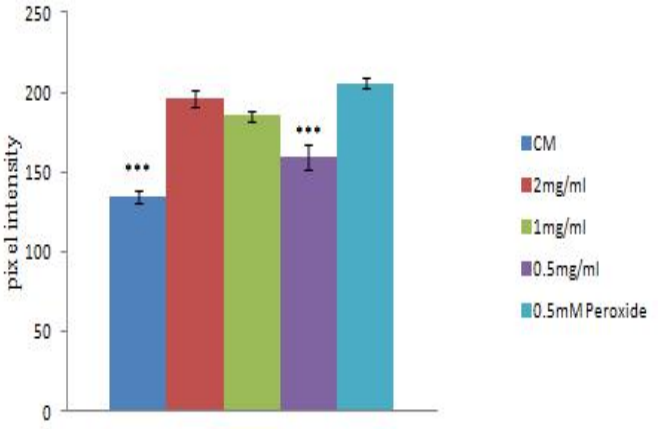


**Figure 2:** Mean pixel intensity of grey image of lenses in various groups. Values are Mean ± S.E.M., *** = $p < 0.001$ compared to untreated (0.5 mM peroxide) group was considered significant, n = 6. Key: CM = culture media only group, 2, 1 and 0.5mg/ml extract treated groups and 0.5 mM peroxide as the untreated group.

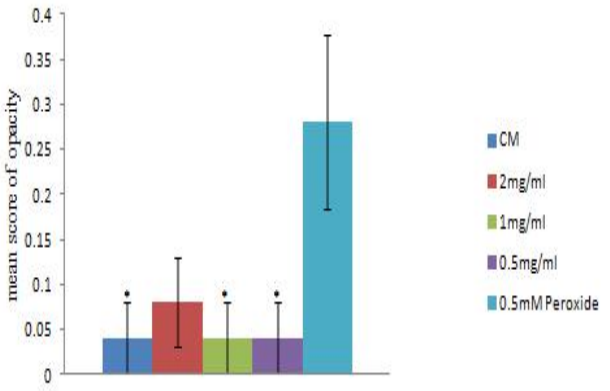


**Figure 3:** Mean score of opacity of lenses in various groups. Values are Mean ± S.E.M., * = $p < 0.05$ compared to untreated (0.5mM peroxide) group was considered significant, n=6. Key: CM = culture media only group, 2, 1 and 0.5 mg/mL extract treated groups and 0.5 mM peroxide as the untreated group.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

**Table 1:** Effect of the Extract of *C. spectabilis* on the Level of Biochemical Parameters of the Lenses

| Treatment | TPC (mg/g) | GSH (mg/g) | SOD (U/mg protein) | MDA(µmol/mg protein) |
|---|---|---|---|---|
| CM | 454.58 ± 59.00* | 19.93 ± 2.20* | 20.23 ± 1.11 | 51.20 ± 0.66* |
| CS 2 | 410.40 ± 50.00 | 16.63 ± 1.03 | 18.10 ± 0.05 | 56.40 ± 0.55 |
| CS 1 | 322.62 ± 21.00 | 15.30 ± 1.19 | 19.73 ± 1.93 | 53.70 ± 1.73 |
| CS 0.5 | 323.00 ± 30.00 | 14.16 ± 0.16 | 18.23 ± 0.16 | 53.70 ± 1.90 |
| P (0.5) | 286.46 ± 29.00 | 12.04 ± 0.03 | 17.43 ± 0.66 | 56.93 ± 0.65 |

Values are Mean ± S.E.M., * = $p < 0.05$ compared to untreated group (P 0.5) was considered significant, n = 6. Key: CM = culture media only group, CS 2, 1 and 0.5 (mg/mL) extract treated groups and P (0.5) the untreated group.

Previous studies have associated some flavonoids and polyphenolic compounds of some plant extracts with anticataractogenic activity. For example, extracts of *Ocinum sanctum, Camelia sinensis* and *Zingiber officinalis* [5]. The reported activity of these extracts was attributed to their antioxidant property. Anticataract activity of lanosterol, a sterol has also been reported.[22] In this study the extract of the plant was found to contain steroids, triterpens, saponins and flavonoids. The observed activity of the extract may be exerted by one or a combination of these phytochemical compounds.

## Conclusion

From the study, it is evident that the extract of *C. spectabilis* exhibits some degree of cataract ameliorative effect especially at a dose of 0.5mg/ml used. Furthermore, the investigation has identified the various classes of phytochemical constituents of the extract. The informations provided could be useful in providing the scientific basis for the reported use of the plant in folk medicine.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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