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Investigation of the Anti-cataract and Antioxidant Activities of *Cnidoscolus aconitifolius* Leaves Extract *In vitro*

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Abstract
Background: Cataract is a major cause of visual impairment and blindness around the world. This study evaluated the *in vitro* antioxidant and anti-cataract activities of *Cnidoscolus aconitifolius* leaves extract and fractions. Antioxidant activities were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiozoline-6-sulfonic acid) (ABTS), total reducing power, and hydrogen peroxide scavenging assays. Anti-cataract potential was evaluated *in vitro* using goat lenses divided into eight groups of different treatments and incubated in artificial aqueous humor at 37 °C for 72 hours. Glucose-induced opacity in the lenses was observed and biochemical indices quantified (catalase, Malondialdehyde (MDA) and total protein in the lens homogenate).
Results: The crude extract and its fractions possess substantial antioxidant activities. The aqueous fraction exhibited the best DPPH radical scavenging activity (IC_{50} value 78.599 µg/ml); while the dichloromethane fraction exhibited the highest ABTS radical scavenging activity with IC_{50} 66.68 µg/ml. The anti-cataract evaluation of crude and fractions at 250 µg/ml showed a significant increase ($p<0.05$) in the total protein and catalase activity compared to the cataract control group. The malonaldehyde level decreased significantly ($p<0.05$) in all the treated groups.

Conclusion: These results suggest that *Cnidoscolus aconitifolius* leaves extract possesses protective actions against oxidative damage and cataract caused by oxidative stress.

Keywords: *Cnidoscolus aconitifolius*, phytochemicals, antioxidants, visual impairment, anticataract activity.

Introduction

Cataract is a visual impairment due to opacification or optical dysfunction of crystallin lens; it affects millions of people around the world and its prevention is vital to the control of global blindness [1, 2]. Statistics from the World Health Organization (WHO) indicate that cataract is the primary cause of about 90% of blindness in people living in the developing world [2]. Cataract surgery involving the removal of lens and correction of eyesight with eye glasses or an intraocular corrective lens remains the most successfully developed treatment option for cataract [1, 3, 4]. However, the cost of surgery and aftermath complications of the surgical procedure remain a burden to both patients and surgeons [5]. Prophylactic measures are constantly desired in the treatment of cataracts.

Extracts from plants contain minerals, primary metabolites and diverse arrays of secondary metabolites with antioxidant activities. These properties have influenced the use of plants for

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medicinal purposes in the primary health care systems of many nations [6, 7]. Many pharmaceutical companies are spending time, money, and other valuable resources in identifying and developing chemicals from plant origin to serve as cost-effective remedies that are affordable to the population [8-11]. These phytochemicals, such as phenolic acids, isoflavones, vitamins, micro-nutrients, carotenoids, and flavonoids are known for their antioxidant activities and can be used in the management of cataract [1, 3, 4, 12].

Oxidative stress-related events, such as chronic hyperglycemia, aging, nutritional deficiencies, trace metals, sunlight, smoking, and certain drugs, have been associated with the high incidence of cataract around the globe [13]. These events have stimulated increasing investigations of suitable antioxidants that are effective in preventing cataracts. A large number of medicinal plants and synthetic compounds have been reported to possess anti-cataract properties [1, 3].

Cnidoscolus aconitifolius, also referred to as spinach tree, belongs to the Euphorbiaceae family. It is a fast-growing vegetative perennial shrub that produces attractive large, dark green leaves. Numerous medicinal benefits have been attributed to the consumption of *Cnidoscolus aconitifolius* [14, 15]. This study was carried out to investigate the anti-cataract and antioxidant activities of *cnidoscolus aconitifolius* leaves extract in an effort to explore the use of natural plant resources in management of cataract.

Materials and Methods

2.1 Chemical Reagents

2,2-diphenyl-2-picryl hydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiozoline)-6-sulfonic acid (ABTS), gallic acid, quercetin, trolox, and L-ascorbic acid were purchased from Sigma (Sigma–Aldrich, Germany). Other reagents and solvents used in this work were of analytical grade and procured from reputable vendors.

2.2 Collection and preparation of plant material

Fresh leaves of *Cnidoscolus aconitifolius* were collected from Mubi North Local Government Area of Adamawa State, Nigeria. The plant material was identified and authenticated in the Department of Plant Science, Modibo Adama University of Technology, Yola.

The fresh leaves of *Cnidoscolus aconitifolius* were separated from undesirable parts, washed and shade-dried in the laboratory at room temperature (28±3 °C) for 14 days. The leaves were pulverized into powder and 200 g of the powder was extracted with methanol (1200 ml x 2) in an airtight container with continuous stirring on a magnetic stirrer for 72 hours. The mixture was sieved using a muslin cloth and filtered using Whatman No. 1 filter. The filtrate was concentrated using a rotary evaporator at 40 ^{o}C and the remaining solvent extract was finally evaporated using an oven at 40 ^{o}C to obtain the solid methanol extract.

2.2 Fractionation of the plant extract

The methanol extract was dissolved in distilled water and partitioned successively in a separatory funnel using n-hexane, dichloromethane and ethyl acetate. For each solvent, the process was repeated thrice and the products were pooled together. The pooled fractions were concentrated in an oven at 40 °C for 48 hours. After concentrating the fractions, their final weights were noted.

2.3 Collection of lenses and induction of cataract

Fresh goat (*Capra aegagrus Hirus*) eyeballs were obtained from Jimeta abattoir immediately after slaughtering the goats and transported to the laboratory using ice bucket. The eyeballs were dissected to obtain the lenses and washed with 0.1 M phosphate buffer saline. The transparency of the lenses was observed and those that met the experimental requirements were used for the experiments.

2.4.0 *In vitro* antioxidant studies

2.4.1 DPPH radical scavenging activity

The DPPH free radical scavenging activity of the extracts was measured according to the method described by Alara *et al*. [16]. Equal volumes of different concentrations of the plant extract (20-100 µg/ml, in methanol) were added to 2 ml solution of DPPH. The same concentrations of gallic acid (20-100 µg/ml) were used as the standard antioxidant. The mixtures were incubated for 30 min at room temperature and absorbance measured at 517 nm using a spectrophotometer.

The percentage inhibition was calculated using the equation below:

$$\% \text{ Inhibition} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

where Abs (control) is the absorbance without extract; Abs (sample) is absorbance with extract or standard.

2.4.2 ABTS radical scavenging activity

The ABTS radical scavenging assay was performed according to the method reported by Alara *et al*. [16]. ABTS and potassium persulfate were dissolved in distilled water to a final concentration of 7 mM and 2.45 mM, respectively. These two solutions were mixed in the ratio 1:1 and allowed to stand in the dark at room temperature (28±3 ℃) for 12 h in order to produce ABTS radical (ABTS•+). Prior to the assay, the ABTS•+ solution was diluted with methanol (1:60 v/v) to obtain an absorbance of 1.08 ± 0.02 at 734 nm; 20-100 µg/ml of the extracts and fractions were prepared in methanol and the diluted ABTS solution (2 ml) was added to the sample or trolox standards and absorbance was measured at room temperature 60 min after mixing.

$$\% \text{ Scavenged (ABTS)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \text{ X } 100.$$

2.4.3 Hydrogen peroxide scavenging activity assay

A solution of hydrogen peroxide (40 mM) in phosphate buffer (50 mM, pH 7.4) was used for the assay. Varying concentrations (20-100 µg/mL) of the extract and fractions of *C. aconitifolius* in phosphate buffer were added to hydrogen peroxide and absorbance was measured at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. L-ascorbic acid was used as standard for comparison. The percentage of hydrogen peroxide scavenging was calculated as follows:

$$\% \text{ Scavenged } (H_2O_2) = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \text{ X } 100$$

2.4.4 Total reducing power assay

The total reducing power of the crude extract and fractions of *C. aconitifolius* was measured using the potassium ferricyanide reduction method as described by Repon *et al*. [17].

2.5. *In vitro* assessment of cataract

The method described by Kurmi *et al*. [18] was used for the *in vitro* assessment of cataract. Goat lenses were incubated at 37 °C in artificial aqueous humor (NaCl 140 mM, KCl 5 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, NaH $(PO_4)_2$ 0.5 mM, $CaCl_2$ 0.4 mM and glucose 5.5 mM, adjusted to pH 7.8). The crude extract and fractions (250µg/ml), as well as penicillin 32 mg and streptomycin 250 mg, were incorporated into the artificial aqueous humor. The setup was incubated for 72 hours.

2.5.1 Experimental design for evaluation of *in vitro* anti-cataract potential

The method described by Kurmi *et al*. [18] was used to induce cataract. Lenses were divided into eight groups (n= 5 in each group) as outlined below:

Group I: Normal lens (Glucose 5.5 mM)
Group II: Lens + Glucose 55 mM only
Group III: Lens + Glucose 55 mM+ Enalapril 5µg/ml
Group IV: Lens + Glucose 55 mM + Crude extract 250 µg/ml
Group V: Lens + Glucose 55 mM + n-Hexane fraction 250 µg/ml
Group VI: Lens + Glucose 55 mM + Dichloromethane fraction 250 µg/ml
Group VII: Lens + Glucose 55 mM + Ethylacetate fraction 250 µg/ml
Group VIII: Lens + Glucose 55 mM + Aqueous fraction 250 µg/ml

2.5.2 Photographic evaluation

Lenses were collected after 72 hours of incubation in the culture media; each lens was placed on wire mesh with the posterior surface touching the mesh and photographed in order to observe the opacity of the lens. The opacity was graded as follows:

0: Absence of opacity
+: Slightly opaque
++: Diffusely opaque
+++: Extensively opaque

2.5.3 Preparation of Lens Homogenate and biochemical assessment

The lenses were homogenized in 0.1 M phosphate buffer saline (pH 7.4) to give 10% homogenate (w/v). The homogenate was centrifuged at 5000 × g for 10 min at 4 °C to obtain a supernatant. After

centrifugation, the collected supernatants were used for the determination of lipid peroxidation level, catalase activity, and total protein.

2.5.4 Lipid peroxidation level

Lipid peroxidation in lens homogenate was measured using the method described by Nieshus and Samuelsson (1986). About 0.1 ml of tissue homogenate was treated with two ml (1:1:1, Thiobarbituric acid 0.37%, 0.25N HCl and 15% Trichloroacetic acetic); the mixtures were incubated in a boiling water bath (95°C) for 15 min, cooled, and centrifuged at 1000 g at room temperature for 10 min. The absorbances of the clear supernatants were measured against a reference blank at 535 nm. The concentration (µmol) of malonaldehyde (MDA) was calculated using the equation below

$$Concentration\ of\ MDA = \frac{\text{Absorbance of test X Total assay volume}}{\text{Extinction coefficient of MDA } (1.56\text{X } 10-5)\ X\ sample\ volume}$$

2.5.5 Catalase assay

Catalase activity in the homogenates was assayed following the procedure described by Kiran and Aruna [19].

2.5.6 Total protein

Protein content was measured by the method of Lowry *et al*. [20]. The protein content was calculated from a standard curve prepared with bovine serum albumin (BSA) and expressed as mg/ml.

2.6 Statistical analysis

The results were expressed as Mean ± Standard Error and the difference between means was analyzed using one-way analysis of variance. Significance was taken at $p<0.05$ using GraphPad prism version 5.01 for Windows, GraphPad Software, San Diego, California, USA.

Results and discussion

Radical-scavenging activities of DPPH, ABTS cation, and hydrogen peroxide, as well as total reducing power, are well-proven parameters for evaluating antioxidant capacity of plant extracts. This study evaluated the antioxidant potentials of *Cnidoscolus aconitifolius* leaf extracts using these parameters. The antioxidant activities of plants are beneficial in the management of diseases [21]. Numerous benefits have been attributed to the use of *C. aconitifolius* leaves. Its uses include the treatment of inflammatory swelling, venereal disease, gout, scorpion stings, and diabetes [22]. Most of these diseases are associated with oxidative stress.

3.1 DPPH radical scavenging activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) is used in many *in vitro* antioxidant studies for evaluating the free radical scavenging potentials of natural compounds [10, 23]. The results of the DPPH scavenging activities of the crude extract and the fractions of *C. aconitifolius* are shown in Table-1. Antioxidant activity, determined by DPPH assay, suggests a concentration-dependent activity for the extract. The aqueous fraction showed similar activity as that of the gallic standards (71.38 - 91.37 %), but significantly increased activity compared to *C. aconitifolius* methanol extract (33.77 ± 1.87 %) at the highest concentration. The aqueous fraction exhibited the highest DPPH radical scavenging activity with IC_{50} value of 78.599 µg/ml, followed by the dichloromethane fraction (87.754 µg/ml). Similar results were obtained by Paredes and coworkers [24] with *C. quercifolius Pohl*, a plant of the same genus as *C. aconitifolius*. The differences observed in the DPPH free radical scavenging activity of the fractions could be attributed to the difference in the constituents of the fraction. The IC_{50} of the crude extract in this study is higher than those of the fractions, hence the fractions possess better radical scavenging potentials than the crude extract.

3.2 ABTS radical scavenging activity

The ABTS radical de-colorization test is a widely used approach to evaluate antioxidant activity [25]. The results of the ABTS radical scavenging activity, shown in Table-2, indicate that the crude extracts and fractions exhibited ABTS radical scavenging activity; at lower concentrations, trolox exhibited a significantly ($p<0.05$) higher ABTS radical scavenging activity than the crude extracts and fractions. However, at 100 µg/ml, the dichloromethane and aqueous fraction showed significantly ($p<0.05$) higher ABTS radical scavenging activity.

Table 1-DPPH radical scavenging activity (%) of methanol extract and fractions of *C. aconitifolius*

Concentration (µg/ml)	Methanol extract	N-hexane fraction	Dichloromethane fraction	Ethylacetate fraction	Aqueous fraction	Gallic Acid
20	1.86 ± 0.94	11.72 ± 0.99^{c}	29.42 ± 0.65^{cde}	13.78 ± 1.86c	21.48 ±0.16^{cde}	45.44 ± 1.38
40	5.59 ± 0.54	11.67 ± 2.57^{c}	35.44 ± 1.71^{cde}	13.22 ± 1.21	22.22 ± 1.65^{cde}	51.52 ± 0.34
60	24.02 ±0.67	24.89 ± 0.81^{e}	32.09 ±0.88^{cde}	13.59 ± 1.94	29.17 ± 1.83e	60.77 ± 1.05
80	25.51± 0.43	39.17 ±0.94^{ce}	47.42 ± 0.76^{ce}	26.82 ± 1.80	53.38 ± 0.66^{cde}	79.14 ± 0.97^{a}
100	33.77 ± 1.87^{b}	42.21 ± 0.48^{c}	45.85 ±1.29^{c}	43.64 ± 1.79^{c}	62.45 ± 1.49^{cdef}	91.37 ± 3.48^{ab}
IC_{50} (µg/ml)	**136.066**	**114.403**	**87.754**	**130.32**	**78.599**	**39.473**

Values are presented as Mean ± Standard Error of Mean, ^{a} significantly ($p<0.05$) higher compared to other values on the same row; ^{b} significantly ($p<0.05$) higher compared to value on the same column; ^{c} significantly ($p<0.05$) higher compared to methanol extract at the same concentration; ^{d} significantly ($p<0.05$) higher compared to N-hexane fraction at the same concentration; ^{e} significantly ($p<0.05$) higher compared to ethyl acetate fraction at the same concentration; ^{f} significantly ($p<0.05$) higher compared to dichloromethane fraction.

Table 2-Percentage ABTS radical scavenging activity of methanol extract and fractions of *C. aconitifolius*

Concentration (µg/ml)	Methanol extract	N-hexane fraction	Dichloromethane fraction	Ethyl acetate fraction	Aqueous fraction	Trolox (Standard)
20	06.50 ± 0.69	01.86 ± 0.30	10.87 ± 0.68	14.99 ± 0.85	07.60 ± 0.39	27.70 ± 0.61
40	10.23 ± 0.46	25.61 ± 0.38	37.78 ± 0.60	14.77 ± 0.52	30.32 ± 0.34	32.79 ± 0.25
60	28.34 ± 0.94	36.36 ± 1.44	49.65 ± 0.92	26.05 ± 1.66	33.74 ± 0.43	52.22 ± 0.94^{a}
80	45.48 ± 1.70	40.10 ± 0.58	52.58 ± 0.1.24	39.15 ± O.81	52.55 ± 1.28	66.79 ± 0.09^{a}
100	46.61 ± 0.93	57.79 ± 1.97^{c}	77.51 ± 0.25^{bc}	44.03 ± 0.47	75.15 ± 8.27c	95.91 ± 0.20^{ac}
IC_{50} (µg/ml)	**99.09**	**84.84**	**66.68**	**113.85**	**70.89**	**28.42**

Values are Mean ± SEM, ^{a} significantly ($p<0.05$) higher compared to methanol extra; ^{b} significantly ($p<0.05$) higher compared to other fractions at the same concentration; ^{c} significantly ($p<0.05$) higher compared to values on the same column.

3.3 Total reducing power

The total reducing antioxidant power assay evaluates the potential of extracts to form the complex with metal atoms, particularly iron and copper. The results in Table-3 represent the total reducing power of the crude extract and fractions of *C. aconitifolius*. The crude extract and fractions showed a concentration-dependent increase in the total reducing power; at higher concentrations (80 and 100 µg/ml), the n-hexane, dichloromethane, ethyl acetate, and aqueous fractions exhibited better reducing power compared to L-ascorbic acid and the crude extract.

3.4 Hydrogen peroxide scavenging activity

The results in Table-4 show the hydrogen peroxide scavenging activity of the crude extract and fractions of *C. aconitifolius* and L-ascorbic acid. The hydrogen peroxide scavenging activity of dichloromethane fraction (IC_{50} 139.86 µg/ml) was comparable to that of L-ascorbic acid (IC_{50} 132.96

µg/ml). The scavenging activity of the crude extract and fractions increased with increasing concentrations of the extracts.

3.5 Photographic observations on the lenses from various treatments

In this study, cataract was induced by incubating lenses in culture media containing high glucose (55mM); this occasioned the formation of superoxide radicals and H_2O_2 leading to oxidative stress [26]. Lens opacification in the control group was visible at 48th hour after incubation. Figure-1a shows a representative of the normal lens incubated with artificial aqueous humor, where the lenses were clear and void of opacity. Figure-1b exemplifies the cataract control lenses incubated with artificial aqueous humor and glucose (55 mM). The lenses showed extensive opacification after incubation. Figure-1c represents the drug control group; the lenses were incubated with artificial aqueous humor, 55mM glucose and standard drug. The physical evaluation showed normal transparent lenses. Figures- 1d, 1e, 1f, 1g, and 1h represent the groups treated with 250 µg/ml crude extract of *C. aconitifolius*, n-hexane, dichloromethane, ethyl acetate, and aqueous fractions, respectively. The physical evaluation of these groups indicates varying effects of the crude extracts and fractions on cataract lenses. Table-5 summarizes the results from the photographic evaluation and indicates the extent the crude extract and fractions of *C. aconitifolius* had on ameliorating cataract induced on lenses by exhibiting reduction in the opacity of the lenses.

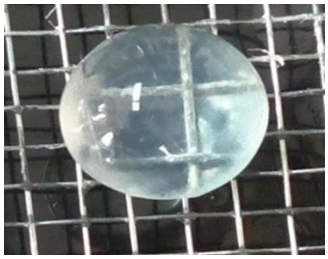

Figure 1a. Lens representing the normal control group

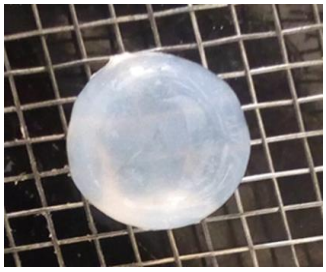

Figure 1b. Lens representing the cataract control group

Figure 1c. Lens representing group treated with enalapril

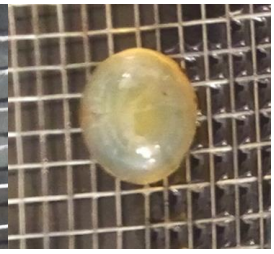

Figure 1d. Lens representing group treated with crude methanol extract

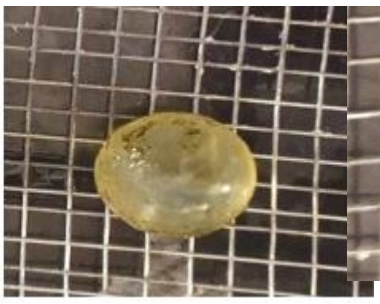

Figure 1e: Lens representing treated with the n-hexane fraction.

Figure 1f. Lens representing group treated with dichloromethane fraction

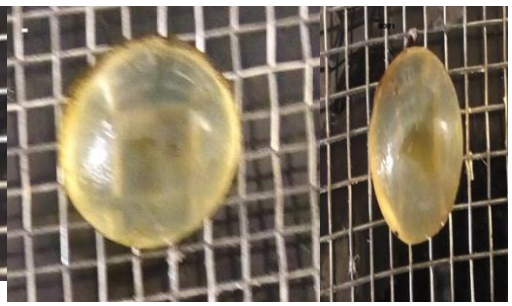

Figure 1g. Lens representing group treated with Ethyl acetate fraction.

Figure 1g. Lens representing group treated with aqueous fraction

Figure 1-Images of lenses after treatment using the crude and fractions of *C. aconitifolius*

Table 3-Total Reducing Power of Methanol Extract and Fractions of *C. aconitifolius*

Concentration (µg/ml)	Methanol Extract	N-hexane fraction	Dichloromethane fraction	Ethyl acetate fraction	Aqueous fraction	L-Ascorbic acid
20	0.747 ± 0.049	1.524 ± 0.200^{abdef}	0.779 ± 0.053	0.698 ± 0.024	0.533 ± 0.025	0.635± 0.026
40	0.634 ± 0.052	1.921 ± 0.022^{abdef}	0.653 ± 0.024	0.690 ± 0.012	0.610 ± 0.003	0.779 ± 0.017
60	0.739 ± 0.022	2.125 ± 0.052^{abdef}	0.749 ± 0.012	0.674 ± 0.009	0.648 ± 0.012	0.828 ± 0.019
80	1.60 ± 0.043^{de}	2.433 ± 0.044^{abdef}	1.006 ± 0.023	0.868 ± 0.023	2.03 ± 0.026^{abde}	1.27 ± 0.027^{e}
100	1.529 ± 0.100	4.496 ± 0.008^{ab}	4.524 ± 0.019^{ab}	4.240 ± 0.144^{ab}	4.524 ± 0.034^{ab}	1.538 ± 0.187

Values are Mean ± Mean ± Standard Error of Mean, [a] significantly ($p<0.05$) increased compared to L-ascorbic acid; [b] significantly ($p<0.05$) increased compared to methanol extract; [c] significantly ($p<0.05$) increased compared to n-hexane fraction; [d] significantly ($p<0.05$) increased compared to dichloromethane fraction; [e] significantly ($p<0.05$) increased compared to ethyl acetate fraction; [f] significantly ($p<0.05$) increased compared to aqueous fraction.

Table 4-Hydrogen Peroxide Scavenging Activity (%) of Crude Extract and Fractions of *C. aconitifolius.*

Concentration (µg/ml)	Methanol extract	N-hexane fraction	Dichloromethane fraction	Ethyl acetate Fraction	Aqueous fraction	L-Ascorbic acid
20	21.66 ± 1.082	20.62 ± 1.190	11.63 ± 0.648^{ce}	17.72 ± 2.198	13.15 ± 0.232	23.33 ± 0.939
40	22.10 ± 1.453	24.84 ± 0.717	17.00 ± 1.316^{abcef}	20.60 ± 0.723	21.44 ± 0.404	23.49 ± 2.230
60	34.47 ± 0.316^{ce}	26.80 ±1.080	31.40 ± 1.711^{e}	24.25 ± 0.967	30.71 ± 0.820^{e}	29.30 ± 1.319
80	34.15 ± 0.251	27.83 ± 0.419	33.33 ± 1.724	29.90 ± 1.300	34.61 ± 0.985^{c}	32.03 ± 1.249
100	37.51 ± 0.459	35.39 ± 0.470	36.11 ± 0.440	35.46 ± 0.6789	35.31 ± 1.100	40.57 ± 1.932
IC_{50} (µg/ml)	**151.50**	**200.886**	**135.11**	**169.035**	**139.86**	**132.96**

Values are Mean ± Mean ± Standard Error of Mean, [a] significantly ($p<0.05$) increased compared to L-ascorbic acid; [b] significantly ($p<0.05$) increased compared to methanol extract; [c] significantly ($p<0.05$) increased compared to n-hexane fraction; [d] significantly ($p<0.05$) increased compared to dichloromethane fraction; [e] significantly ($p<0.05$) increased compared to ethyl acetate fraction; [f] significantly ($p<0.05$) increased compared to aqueous fraction.

Table 5-Degree of Lens Opacification down the Control and Treatment groups

Groups	Grading
Normal control	0
Cataract control	+++
Drug control (enalapril)	0
MCAT	++
HCAT	+
DCAT	0
ECAT	+
ACAT	+

Key: 0: Absence of opacity; +: Slightly opaque; ++: Diffusely opaque; +++: Extensively opaque. MCAT: crude methanol extracts of C. aconitifolius fractions; HCAT: n-hexane C. aconitifolius fractions; ECAT: ethyl acetate C. aconitifolius fractions; DCAT: dichloromethane C. aconitifolius fraction and ACAT: aqueous C. aconitifolius fraction.

Total protein, lipid peroxidation, and catalase activity

The polyol pathway is the primary mediator of diabetes-induced oxidative stress in the lens due to the formation of superoxide radicals and H_2O_2 [26, 27]. Accumulation of polyols in the lens results in an osmotic imbalance and induces stress in the endoplasmic reticulum (ER), the principal site of protein synthesis that leads to the generation of free radicals. Likewise, elevated glucose levels cause ER stress by initiating an unfolded protein response (UPR) that generates reactive oxygen species (ROS) and causes damage to lens fibers [28]. Antioxidants have the ability to inhibit oxidations caused by ROS, using mechanisms such as trapping radicals by donating a hydrogen atom to one radical and receiving an electron from another radical to form stable non-radical products and metal chelating which converts metal prooxidants into stable products [29, 30].

The total protein and MDA levels of both the control and treated lenses clearly showed that cataract was induced with 55 mM glucose (Table-6). The crude extract of *C. aconitifolius*, as well as the fractions, showed increased total protein content compared to the cataract control lenses. The total protein (mg/ml) of the normal lenses (6.254 ± 0.214) is significantly higher ($P<0.05$) than that of the cataract control (3.968 ± 0.032), while no significant difference ($P<0.05$) existed between the normal control and the drug control (5.987 ± 0.075). The crude extracts of *C. aconitifolius* treated group and its fractions exhibited significantly increased ($P<0.05$) protein content than the cataract control. The standard drug Enalapril also showed increased total protein content. The conservation of protein levels demonstrates that the crude extract and its aqueous fraction might have exerted anti-cataract effect by promoting protein synthesis or preventing their degradation in the lens (Moreau and Kind, 2012). The results of the lipid peroxidation levels indicated the MDA levels of cataract control (4.642 ± 0.494), normal control (0.536 ± 0.016) and the drug control (0.391 ± 0.036). The groups treated with crude methanol extracts of *C. aconitifolius* (MCAT), n-hexane (HCAT) and ethyl acetate (ECAT) fractions showed significantly ($P<0.05$) higher MDA level: 2.267 ± 0.330, 3.509 ± 0.265, and 1.874 ± 0.058, respectively, compared to the drug control (0.391 ± 0.036) and the normal control (0.536 ± 0.016) (Table-6). However, there was a decreased level of peroxidation products in the groups treated with dichloromethane (DCAT) and aqueous (ACAT) fractions. The results showed that the n-hexane fraction was ineffective in preventing lipid peroxidation. But the dichloromethane, ethyl acetate and aqueous fractions were effective in the prevention of lipid peroxidation and delayed the onset of cataract.

Catalase is an important component of the innate enzymatic defenses of the lens. It is responsible for the detoxification of significant amounts of H_2O_2, thereby decreasing the extent of cellular damage inflicted by the products of lipid peroxidation [18]. Reduction in catalase activities in tissues has been associated with accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes. The results (Table-6) show a significantly low ($P<0.05$) catalase activity in the cataractous lenses as compared to the enalapril-treated lenses and lenses treated with crude extract and fractions from *Cnidoscolus aconitifolius*. The catalase activity increased significantly in the group treated with enalapril in comparison to the cataract induced lenses. Likewise, all the fractions from *C. aconitifolius* significantly increased catalase activity in a manner

similar to the standard drug. This corroborates the results obtained from previous studies and establishes that phytochemicals commonly found in fruits and vegetables have complementary and overlapping mechanisms of actions including, antioxidant activity and scavenging of free radicals [31].

Table 6-Effects of *Cnidoscolus aconitifolius* on Total Protein, Catalase and Malonaldehyde Level of Normal and Cataract Goat lens

Groups	Total protein (mg/ml)	Catalase activity (µmol/min/mg protein.)	MDA level (µmol/ml)
Normal control	6.254 ± 0.214^{befgh}	799.1 ± 9.421^{bdefg}	0.536 ± 0.016
Cataract control	3.968 ± 0.032	561.2 ± 21.88	4.642 ± 0.494^{acdfgh}
Drug control	5.987 ± 0.075^{befg}	746.7 ± 12.69^{bd}	0.391 ± 0.036
MCAT (250µg/ml)	6.033 ± 0.472^{befg}	589.5 ± 12.18	2.267 ± 0.330^{acfh}
HCAT (250µg/ml)	4.591 ± 0.087	722.8 ± 10.80^{bd}	3.509 ± 0.265^{acdfgh}
DCAT (250µg/ml)	4.688 ± 0.106	691.5 ± 12.06^{bd}	0.697 ± 0.143
ECAT (250µg/ml)	4.935 ± 0.239^{b}	715.2 ± 8.911^{bd}	1.874 ± 0.058^{acf}
ACAT (250µg/ml)	5.111 ± 0.113^{b}	695.5 ± 21.18^{bd}	0.719 ± 0.076

MCAT: crude methanol extracts of C. aconitifolius fractions; HCAT: n-hexane C. aconitifolius fractions; ECAT: ethyl acetate C. aconitifolius fractions; DCAT: dichloromethane C. aconitifolius fraction and ACAT: aqueous C. aconitifolius fraction. Values are presented as mean ± SEM, [a] significantly ($P<0.05$) higher than normal control; [b] significantly ($P<0.05$) higher than cataract control; [c] significantly ($P<0.05$) higher than drug control; [d] significantly ($P<0.05$) higher than MCAT; [e] significantly ($P<0.05$) higher than HCAT; [f] significantly ($P<0.05$) higher than DCAT; [g] significantly ($P<0.05$) higher than ECAT; [h] significantly ($P<0.05$) higher than ACAT.

Conclusion

This study concluded that *Cnidiscolus aconitifolius* has the ability to ameliorate various pathological conditions such as cataract occasioned by oxidative stress.

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