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Original Article

# Evaluation of Lens Aldose Reductase Inhibitory and Free Radical Scavenging Potential of Fractions of *Lonchocarpus cyanescens*: Potential for Cataract Remediation

**Emmanuel Oladipo Ajani[1]*, Saheed Sabiu [1,2], Kuburat Temitope Odufuwa[3], Taiwo Bashir Ibrahim[1] and Bamidele Adewale Salau[4]**

**ABSTRACT**

**Introduction:** Current research effort at discovering effective anticataract agent is focus on evaluating Aldose reductase inhibition (ARI) capacities of medicinal plants and plant extracts. The present study was aimed at investigating the *in vitro* ARI activity of fractions of *Lonchocarpus cyanescens* leaves on partially purified AR from goat lens. **Methods:** Phytochemical constituents of the leaves were screened for using aqueous and methanolic extract while *in vitro* free radical scavenging and ARI activities of the fractions were evaluated using partially purified aldose reductase. Kinetics of the enzyme in the presence of fractions of the leaves was then compared using Lineweaver-Burk plot. **Results**: Phenol, flavonoid, tannins and phlobatannins were detected in both extracts. All the fractions inhibited AR significantly but this was strongest with aqueous ($IC_{50}$, 0.06 ± 0.02 $mM^{-1}$ and chloroform fractions ($IC_{50}$, 0.09 ± 0.01 $mM^{-1}$). Ethyl acetate, hexane and methanol fraction showed competitive inhibition whereas the aqueous and chloroform fractions showed mixed inhibition with a different $V_{max}$ and $K_m$ when compared with DL-glyceraldehyde. All the fractions also showed antioxidant capacities but this was also strongest with aqueous fraction. **Conclusion:** The study confirms the ARI and antioxidant capacity of *Lonchocarpus cyanescens* which may be attributed to its phenolic constituents and whose extraction is solvent dependent. The plant may therefore serve as a base for the development of anticataract agent.

**Key words:** Aldose reductase, Cataract, Free radical, Medicinal plant, Phytochemical constituents.

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## INTRODUCTION

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." or reactive oxygen species (ROS). Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction.[1,2] Overall, free radicals have been implicated in the pathogenesis of various diseases and particularly diabetes and its secondary complication especially cataract. Cataract is opacity of the lens that interferes with vision, and is the most frequent cause of visual impairment worldwide, especially for the elderly. It is the leading cause of blindness and contributes to 50% of blindness worldwide.[3] The only present remedy for cataract is surgery. Free radicals that have been implicated in cataract formation include superoxide anion ($O_2^{-}$), nitric oxide (NO), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals (OH·). Superoxide anionin itself is not highly toxic but it may react with other molecules, such as NO, yielding more reactive compounds.[4] An excess of NO, produced by inducible nitric oxide synthases (iNOS) upon stimulation, is thought to cause cell injury by nitrosative stress and this may occur in certain diseases. In the eye, NO contributes to allergic conjunctivitis,[5] glaucoma,[6] diabetic retinopathy,[7] and also cataract.[8,9] OH·, another highly reactive free radical has been shown to contribute to lens crystalline modification.[10] Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating.

In addition to hyperglycemia, diabetes mellitus is usually accompanied by increased generation of free radicals or impaired antioxidant defenses. Several hypothesis have been put forth to explain the genesis of free radicals in diabetes. Forma-

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tion of excess superoxide radicals by the mitochondrial transport chain during hyperglycemia has been reported to be the initial factor.[11] Other major contributing factors include increased glucose flux through the polyol pathway, advanced glycated end products, protein kinase-C activation and increased flux through the hexosamine pathway.[12] Of importance to this study is the polyol pathway and especially the role of aldose reductase enzyme in the pathway. Aldose reductase is the first and rate-limiting enzyme in the polyol pathway and reduces glucose to sorbitol utilizing NADPH as a cofactor. Sorbitol is then metabolized to fructose by sorbitol dehydrogenase.[13] Normally, the polyol pathway represents a minor route of glucose utilization, accounting for <3% of glucose consumption. However, in the diabetic state, the activity of this pathway is substantially increased and could represent up to 30% of total glucose consumption. Sorbitol does not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in the microvascular complications of diabetes such as peripheral neuropathy, nephropathy, retinopathy, and cataracts.[12]

Aldose reductase enzyme and especially its inhibition by aldose reductase inhibitors (ARIs), has been gaining attention over the last years from the pharmaceutical community, as it appears to be a promising pharmacotherapeutic target. Several authors have studied and reported on a number of structurally diverse naturally occurring and synthetic AR inhibitors that have proven to be effective for the prevention of diabetic complications in experimental animals, as well as in clinical trials.[14,15] Although, some synthetic aldose reductase inhibitors (ARIs) have been developed as drug candidates, however, virtually all have not been successful in clinical trials due to adverse pharmacokinetic properties, inadequate efficacy, and toxic side effects. The use of medicinal plants, plant extracts or plant-derived pure chemicals to treat human ailments is an important alternative therapeutic approach. The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research.[16]

*Lonchocarpus cyanescens* Benth., is a deciduous scan dent shrub.[17-19] Its common name is West Africa indigo. In Nigeria, the plant is known as 'elu' (Yoruba), 'anunu' (Ibo), 'talaki' (Hausa), 'suru' (Tiv) and 'ebelu' (Edo).[20] The plant has alternate leaves and flat fruits which are 1 - 5 seeded, oblong pod pointed at both ends.[19,21,22] The aerial parts yield an indigo, which is a useful colourant for cloth dyeing [adire/gara] in West Africa since ancient times[23] Some of the reported medicinal uses of the plant include poultice to treat skin diseases and ulcers (leaves and roots); possible cure for leprosy (leaves and roots); laxative (leaves); intestinal disorders and dysentery (leaf sap); diarrhea, aphrodisiac, veneral diseases and athritis (decoction of leafy twigs and roots); yaws and sores (ground root).[5] Bioactivity effects of *Lonchocarpus cyanescens* have also been demonstrated in its anti-inflammatory, anti-arthritic and its relief on ulcer.[20] The present study is aimed at evaluating the aldose reductase inhibitory and the free radical scavenging activity of *Lonchocarpus cyanescens*.

## MATERIALS AND METHODS

Quercetin, 2,2-diphenyl-1-picryl hydrazine (DPPH), TCA, Glacial acetic acid, NADPH, ferrozine, napthylenediamine, dihydrochloride, 1,10-phenanthroline, sulfanilic acid, rutin and 2-mercaptoethanol were obtained, from Sigma–Aldrich Co. (St. Louis, MO, USA). All other solvents and chemicals used were of analytical grade and were obtained from commercial sources.

### Plant Materials

Whole plant of *Lonchocarpus cyanescens* was collected from a botanical garden in OkeOdo area of Ilorin, Kwara State in July 2015. The plant was identified at the herbarium of Plant Biology, Kwara State University, Malete, Nigeria where a voucher specimen (KS/PLH/ASA4/0/002/101) was deposited. The leaves of the plant were then shade dried for two (2) weeks and then pulversised with a local kitchen blender.

### Preparation of Extract and Fractions

Exactly 100 g of the pulverized leaves was macerated in 500 ml of methanol for 7 days. It was then sieved over a muslin cloth first and again with filter paper. The residue was again macerated in another 500 ml of methanol for another 7 days and then filtered as above. The filtrates were combined and concentrated using a rotary evaporator under vacuum (model RE, 300 Yamato, Japan). The concentrated product was further dried over a water bath at 40$^0$C and weighed. The dried extract was then fractionated by suspending it in distilled water. Hexane was added to the suspension in ratio 1:2, shook well and allowed to stand for about fifteen minutes until two layers were formed. The hexane layer was removed and more hexane was added to the aqueous layer. The process was repeated once, and then a colorless hexane layer was seen. The two hexane layers were combined and dried to obtain the hexane fraction. The procedure was repeated with the aqueous layer using chloroform and ethyl acetate respectively. Each fraction obtained including the aqueous fraction was then collected and dried. Aqueous fraction was dried by lyophilizing for a total of 72 hours. The fractions obtained with othersolvents were recovered using rotary evaporator under vacuum. The evaporation process was conducted at 40°C to minimize any possible degradation of the phytochemicals in the samples. The weight of the dried fractions were calculated and the samples were then stored in a desiccator for further use.

### Isolation and Partial Purification of Goat Eye Lenses

Eye ball was removed from goat immediately after sacrifice and stored in ice-cold container. Lenses were removed by lateral incision of the eye, washed with ice-cold distilled water and kept cold. The lenses were homogenized in 10 volumes of 100 mM ice- cold potassium phosphate buffer, pH6.2 and centrifuged at 15,000 xg for 30 minutes at 4$^0$C. The resulting supernatant was used as the source of aldose reductase. Saturated ammonium sulphate (100%) was added to the supernatant from the homogenate to reach 40% saturation and then allowed to stand for 15 min with occasional stirring to ensure the completeness of precipitation. It was then centrifuged and the precipitate was discarded. The same procedure was repeated for the resulting supernatant using 50% and 75% ammonium sulphate saturations. The final supernatant was used as the partially purified aldose reductase.

### Aldose Reductase Assay

The method of Hayman and Kinoshita[24] was used to assay for aldose reductase (AR) activity. Enzyme specific activity was calculated as IU/mg protein and this was defined as activity of the enzyme that can produce 1µmol $NADP^+$ from NADPH in 1 min.[25]

### Inhibition Study

Varied concentrations (0.4-2.8 mg/ml) of the methanolic extract of the leaves and its fractions were prepared in triplicate. Exactly 100 µl of concentration prepared was then added to the assay mixture and incubated for 5-10 minutes. The reaction was initiated with the addition of NADPH. The absorbance was then read at 340 nm at the beginning and at the end of 30 minutes. The per cent inhibition (%ARI) of the extract/fraction was then calculated as:

$$\% \text{ ARI} = \frac{\Delta \text{ absorbance (negative control)} - \Delta \text{ absorbance (fraction)}}{\Delta \text{ absorbance (negative control)}} \times 100$$

The AR activity in the absence of inhibitor was considered as 100%. The concentration of each test sample that gives 50 % inhibition ($IC_{50}$) was then estimated. A negative control was prepared using 5% DMSO in phosphate buffer (pH6.2).

## Determination of Kinetic Parameters

The kinetic studies of inhibitory activity of the fraction against aldose reductase of different fractions were analyzed using the Line weaver-Burk plot.

## Estimation of Lens Protein Concentration

The protein determination was carried out using the Stoschck,[26] modified method of Lowry *et al.*[27]

## Assay for Free Radical Scavenging Activity

## DPPH Radical Scavenging Assay

The DPPH (1, 1- diphenyl-2-picryl hydrazyl) radical scavenging method was used to evaluate the antioxidant property of the plant. The antioxidant activity of each sample was expressed in terms of $IC_{50}$, and this was calculated from the graph of inhibition percentage against fraction concentration. The assay was carried out according to the method of Hemalatha *et al.*,.[28] DPPH (0.1 mM) was prepared in methanol and 1.0 ml of its solution was mixed with 1.0 ml of extract/fractions prepared in methanol at different concentrations (20, 40, 60, 80 and 100 µg/ml). The mixture was shaken well and incubated at room temperature for 30 minutes and absorbance was measured at 517 nm using a UV- spectrophotometer. All the experiments were performed in triplicate and the mean was taken. Scavenging activity was calculated from control sample absorbance using the following equation:

DPPH- Scavenging capacity (%) = $[(A^0\text{control} - A^0\text{sample}/A^0\text{control}) \times 100]$

Ascorbic acid was used as positive control. $IC_{50}$ values (concentration of extract/fraction required to reduce 50% of DPPH radical) were estimated from the graph and compared.

## Metal Chelating Activity

This was carried out following the method of Decker and Welch. [29] The extract/fraction (0.5 g) was mixed with $FeCl_3$ (2 mM) and ferrozine (0.2 ml) in a test tube, and the total volume was diluted with methanol (2 ml). The mixture was vigorously shaken and left standing for 10 minutes at room temperature. The absorbance of the solution was measured spectrophotometrically at 562 nm after the mixture had reached equilibrium. EDTA was used as positive control and the percent inhibition of ferrozine-$Fe^{2+}$ complex was calculated using the formula stated below.

- Percent Scavenging = $[(A^0\text{control} - A^0\text{sample})/ A^0\text{control}] \times 100$
- Where: $A^0$control = absorbance of ferrozine- $Fe^{2+}$ complex

$A^0$ sample = absorbance of test compound

## Hydroxyl Radical Scavenging Activity

This was carried out according to the method described by Yu *et al.*,[30] The reaction medium was made up of 60 µl of 1 mM $Fecl_3$, 90 µl of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M $H_2O_2$, and 1.5 ml of various concentration of each fractions/methanol extract. Reaction mixture was kept at room temperature for 5 min incubation and absorbance was then measured at 560 nm using spectrophotometer. The concentration of the individual sample required to neutralize 50% hydroxyl radicals were considered as $IC_{50}$ values.

## Nitric Oxide Radical Scavenging Activity

Method previously described by Garrat[31] was used for estimating nitric oxide radical scavenging activity. The reaction mixture containing 2 ml of 10 mM sodium nitroprusside, 0.5 ml of phosphate buffer saline (pH 7.4) and 0.5 ml of plant extract/fraction was incubated at $25^0$C for 2 hr 30 min. After incubation time, 0.5 ml of reaction mixture was mixed with 1 ml of sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated for 5 min. This was followed by addition of 1 ml napthylenediamine dihydrochloride (0.1% w/v). The mixture was incubated at room temperature for 30 min. The absorbance was measured at 560 nm using UV–VIS spectrophotometer. The amount of sample required to scavenge 50% nitric oxide radicals generated in the control set were calculated as $IC_{50}$.

## Hydrogen Peroxide Scavenging Activity

The assay method described by Ruch *et al.*,[32] was used to determine the ability of plant extracts/fraction to scavenge hydrogen peroxide. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The extract/fraction prepared in distilled water was mixed with 0.6 ml of hydrogen peroxide solution (40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing plant extract/fraction in phosphate buffer without hydrogen peroxide. The absorbance of hydrogen peroxide (40 mM) without plant extract was considered as control (100%). The concentration of plant extract/fraction required to scavenge 50% hydrogen peroxide was calculated as $IC_{50}$.

## Reducing Power Assay

This was determined by the previously described method.[33] The reaction mixture containing 0.75 ml of various concentrations of plant extract/fraction, 0.75 ml of phosphate buffer (0.2 N, pH 6.6) and 0.75 ml of potassium hexacyanoferrate [$(K_3Fe(CN)_6$] (1% w/v) was incubated at $50^0$C in water bath for 20 min. The reaction was stopped by the addition of 0.75 ml trichloroacetic acid (10%) and then centrifuged for 10 min at 800 rpm. The supernatant (1.5 ml) of the individual reaction mixture was collected in different clean tubes and was mixed with 1.5 ml of distilled water followed by addition of 0.1 ml ferric chloride (0.1% w/v) and kept for 10 min. The absorbance of reaction mixture was measured at 700 nm as the reducing power. The absorbance of control was considered as 100% of $Fe^{3+}$ ions and $IC_{50}$ values were determined as the concentration of plant extract/fraction required to inhibit 50% reduction of $Fe^{3+}$ ions.

## Phytochemical Screening

The methanolic and aqueous extracts of the plant was subjected to different chemical tests for the detection of the plant phytoconstituents using standard procedures.[34-36]

## Statistics

The experimental results obtained were expressed as mean values of three replicates. Linear regression analysis was used to calculate the $IC_{50}$ values. Data are expressed as mean ± SEM of 3 replicates and were subjected to one way analysis of variance (ANOVA) followed by Duncan Multiple Range Test to determine significant differences in all the parameters. Values were considered significantly different at $p<0.05$.

# RESULT

Preliminary phytochemical screening carried out on the methanolic and aqueous extracts of *Lonchocarpus cyanescens* revealed the presence of

phenols, phlobatannins, flavonoids and tannins in both extracts. Saponin, cardiac glycosides, quinones and alkaloids were however found only in the aqueous extract (Table 1). When the yield of the fractions were compared (Table 2), aqueous fractionyield was the highest (18.20%) while chloroform fraction yield was the lowest (5.10%).

Comparing the effect of incubation of the lens aldose reducatse, results obtained (Figure 1) indicates that all the fractions inhibited lens aldose reductase activity to various degree. The figure showed that, the percentage inhibition increases with the concentration of the fraction until a peak was reached after which further increase in concentration of sample does not result in increase in percentage aldose reductase inhibition (except for hexane fraction). Table 3 is the profile of the concentration of individual fraction required to achieve 50% AR inhibition. Both aqueous and methanolic fractions showed strong aldose reductase inhibition with $IC_{50}$ of 0.06 ±0 0.02 and 0.09 ± 0.01 mg/ml respectively. All other fractions showed mild aldose reductase inhibition with ethylacetate fraction showing the weakest inhibition with $IC_{50}$ of 2.29 ± 0.03 mg/ml. In this study, the Kinetic study was performed for all the fractions in order to understand exactly the mode of inhibition. Data from the study indicates that ethyl acetate, hexane and methanol fraction showed competitive inhibition. The Lineweaver-Burk plot (Figure 2) and the kinetic parameters obtained (Table 4) showed that although the aldose reductase $V_{max}$ obtained when these fractions were incubated with Dl-glyceraldehyde (0.136 ± 0.041, 0.066 ± 0.014 and 0.052 ± 0.023 µm NADPH oxidised/hr/100 mg protein for ethyl acetate, hexane and methanol respectively) were significantly ($p<0.05$) reduced from the control value (0.830 ± 0.031 µm NADPH oxidised/hr/100 mg protein), the $K_m$ were not different significantly ($p>0.05$) different from that of the substrate glyceraldehyde (6.613 ± 0.642, 6.313 ± 0.052, 5.520 ± 0.619 $mM^{-1}$ for ethyl acetate, hexane and methanol respectively). In aqueous and chloroform fraction, the valueof the $V_{max}$ (0.136 ± 0.042 and 0.038 ± 0.012 µm NADPH oxidised/hr/100 mg protein respectively) and $K_m$ (17.900 ± 0.642 and 0.992 ± 0.031 $mM^{-1}$ obtained for aqueous and chloroform fraction respectively) differs significantly ($p<0.05$) when compared with DL-glyceraldehyde value (0.830 ± 0.031 µm NADPH oxidised/hr/100 mg protein for $V_{max}$ and 6.413 ± 0.012 $mM^{-1}$ for $K_m$) which indicates that these fraction showedmixed inhibition.

Table 5 is the result of the free radical scavenging activities of the fractions of *Lonchocarpus cyanescens*. Strongest DPPH scavenging activity was shown by the aqueous fraction with an $IC_{50}$ of 0.050 ± 0.009 mg/ml). All other fractions showed mild DPPH scavenging activity with hexane fraction showing the weakest ($IC_{50}$, 0.139 ± 0.031 mg/ml). Similarly, the metal chelating activity was highest with aqueous fraction (88.6%) and weakest with hexane fraction (18.1%). Results of the hydroxyl radical scavenging activity also showed that the $IC_{50}$ of 0.664 ± 0.039 mg/ml obtained with aqueous fraction was the lowest and was followed by $IC_{50}$ of 0.861 ± 0.098 mg/ml showed by methanol fraction. Hexane fraction showed the least hydroxyl radical scavenging activity with $IC_{50}$ of 1.963 ± 0.041 mg/ml. A similar pattern was seen when the hydrogen peroxide and NO scavenging activities were compared. Result of the reducing power activity also showed that the aqueous fraction elicits the highest reducing power ($IC_{50}$, 0.211 ± 0.003 mg/ml) while the hexane fraction also elicits the least reducing power ($IC_{50}$, 0.613 ± 0.001 mg/ml).

## DISCUSSION

Preliminary qualitative test is useful in the detection of plant bioactive principles and subsequently may lead to drug discovery and development.[37] Our study indicates that *L. cyanescens* leaves contain different phytochemicals with diverse biological properties. In a previous study with *L. cyanescens,* Dorcas *et al.*,[20] reported the presence of phlobatannins (methanolic leave extract), steroid (hexane leave extract), flavonoid

**Table 1: Classes of phytochemicals Detected in of *Lonchocarpus cyanescens* Leaves in aqueous and methanolic extracts**

| Phytochemical constituents' | Aqueous extract | Methanolic extract |
|---|---|---|
| Phenols | + | + |
| Phlobatannins | + | + |
| Tannins | + | + |
| Flavonoids | + | + |
| Saponins | + | - |
| Alkaloids | + | - |
| Anthocyanins | - | - |
| Cardiac glycosides | + | - |
| Terpenoids | - | - |
| Quinones | + | - |

Note: + denotes detected and – denotes not detected.

**Table 2: Yield of extract/ fraction of *Lonchocarpus cyanescens* Leaves (n=3 ± SEM)**

| Extract/ Fraction | Yield (%) |
|---|---|
| Methanol | 11.51 |
| Aqueous | 18.20 |
| Ethylacetate | 16.11 |
| Hexane | 6.71 |
| Chloroform | 5.10 |

**Table 3: $IC_{50}$ (mg/ml) of fractions of *Lonchocarpus cyanescens* leaves on aldose reductase activity**

| Fraction | $IC_{50}$ (mg/ml) |
|---|---|
| Chloroform | 1.03 ± 0.01 |
| Methanol | 0.09 ± 0.01 |
| Ethyl acetate | 2.29 ± 0.03 |
| Aqueous | 0.06 ± 0.02 |
| Hexane | 1.17 ± 0.02 |

- All values are expressed as mean ± SD, n=3.
- Values in the same column with similar superscripts are not significantly different from each other.

**Table 4: Kinetics parameters of aldose reductase enzyme in the presence of different fractions of Lonchocarpus cyanescensleaves**

| Extract/fraction | Vmax (µm NADPH oxidised/hr/100 mg protein) | Km X $10^{-3}$ mM |
|---|---|---|
| DL- glyceraldehyde | $0.830 \pm 0.031^{a}$ | $6.313 \pm 0.012^{a}$ |
| DL- glyceraldehyde + methanol | $0.052 \pm 0.023^{b}$ | $5.520 \pm 0.619^{a}$ |
| DL- glyceraldehyde + chloroform | $0.038 \pm 0.012^{c}$ | $0.992 \pm 0.031^{c}$ |
| DL- glyceraldehyde + hexane | $0.066 \pm 0.014^{d}$ | $6.313 \pm 0.052^{a}$ |
| DL- glyceraldehyde + ethylacetate | $0.139 \pm 0.032^{e}$ | $6.163 \pm 0.137^{a}$ |
| DL- glyceraldehyde + aqueous | $0.136 \pm 0.042^{e}$ | $17.900 \pm 0.642^{d}$ |

- All values are expressed as mean ± SD, n=3.
- Values in the same column with similar superscripts are not significantly different from each other.

**Table 5: Free radical scavenging activity of extract and fractions of Lonchocarpus cyanescens leaves**

| Extract/Fractions | DPPH $IC_{50}$ (mg/ml) | Metal chelating activity | OH $IC_{50}$ (%) | $H_2O_2$ $IC_{50}$ (mg/ml) | Reducing power $IC_{50}$ (mg/ml) | NO $IC_{50}$ (mg/ml) |
|---|---|---|---|---|---|---|
| Methanol | 0.089 ± 0.021[a] | 51.8 | 0.861 ± 0.098[a] | 0.516 ± 0.001[a] | 0.401 ± 0.011[a] | 1.011 ± 0.021[a] |
| Aqueous | 0.050 ± 0.009[b] | 88.6 | 0.664 ± 0.039[b] | 0.603 ± 0.012[b] | 0.211 ± 0.003[b] | 0.538± 0.009[b] |
| Ethylacetate | 0.988 ± 0.054[a] | 39.6 | 0.974 ± 0.063[a] | 0.905 ± 0.001[c] | 0.361 ± 0.008[c] | 1.260 ± 0.014[c] |
| Chloroform | 0.109 ± 0.061[a] | 28.3 | 1.789 ± 0.069[c] | 1.726 ± 0.004[d] | 0.332 ± 0.007[c] | 1.968 ± 0.100[d] |
| Hexane | 0.139 ± 0.031[a] | 18.1 | 1.963± 0.041[c] | 1.631 ± 0.001[d] | 0.613 ±0.011[d] | 2.613 ± 0.091[e] |

- All values are expressed as mean ± SD, n=3.
- Values in the same column with similar superscripts are not significantly different from each other.

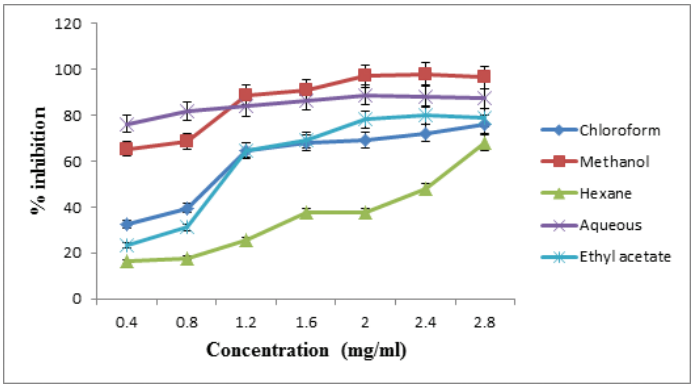


**Figure 1:** *Inhibitory effect of fractions of Lonchocarpus cyanescens Leaves on the specific aldose reductase activity.*

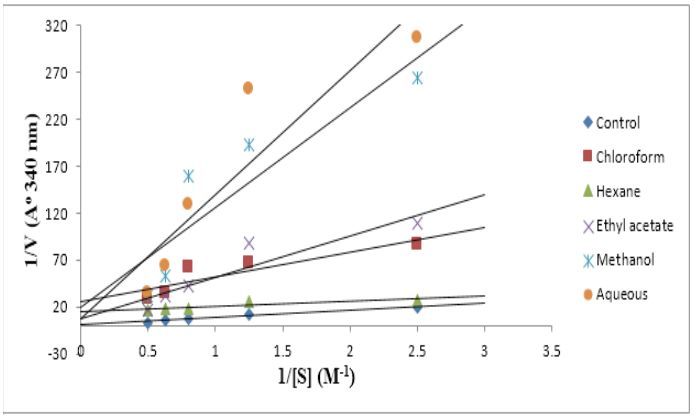


**Figure 2:** *Lineweaver-Burk plot showing the inhibitory effect of fractions of Lonchocarpus cyanescens Leaves on the aldose reductase activity.*

(methanolic leave residue), saponin (methanolic stem extract), tannin and cardiac glycoside (hexane stem extract), phlobatanin (methanolic root extract) and terpenoid (methanolic stem interpahase). In the present study, we detected the presence of phenol, phlobatannin, flavonoid and tannin in both aqueous and methanolic leave extract of the plant while saponin, alkaloids, cardiac glycosides and quinones were detected only in the aqueous extract. More of the phytochemicals were detected in the aqueous extract than the methanolic extract indicating that the efficacy of solvents in extracting the bioactive principles of the plant may correlate with solvent polarity and that it is highest in the most polar solvent. The presence of these phytochemicals in the leaves of *L. cyanescens* may form the basis for its inclusion in Traditional Medical Practices.[38-40] Phenolic compounds are especially common in leaves, flowering tissues and woody parts, such as stems and barks. Reports have implicated phenolic compounds as having health beneficial properties. This is because the compound is able to inhibit aldose reductase and xanthine oxidase enzymes.[41] Reports in the literature indicates that the most widely used solvents for extracting phenolic compounds are water, ethanol, methanol, acetone, and their water mixtures.[42-44] Thepresent study is in agreement with these reports.

When the yields of the fractions were compared, result from the present study indicates that the yield of the aqueous fraction was the highest followed by methanol, ethylacetate, chloroform and hexane in that order. The result suggests that the major phytochemicals in *L. cyanescens,* leaves were mostly high in polarity and soluble in water. This is similar to the observation of Mohd Farhan *et al.*,[45] with *Orthosiphon stamineus.* Although both water and methanol contain hydroxyl group that can form hydrogen bonding with the solute, water may be more effective in extracting the solute because it has higher polarity and shorter chain.[46] These characteristics of water improved its capability to extract the polar compounds. This thus explains the significant difference observed between water and methanol fraction yield. The difference in yields for other solvents may be due to other factors including phytochemicals in plants, extraction temperature, extraction time and solvent to solid ratio.

Result of this study also indicates that all the fraction of *L. cyanescens* leaves investigated in this study this study showed significant aldose reductase inhibitory activity The ARI capacity was seen to be strongest with aqueous and methanolic fraction but weakest with ethylacetate fraction. Chloroform and hexane fraction showed mild AR inhibitory activity. In an attempt to develop potent, safe, and new ARI agents from natural sources, many plant materials and isolated phytoconstituents have been tested for ARI activity in both *in vivo* and *in vitro* models. Halder *et al*[25] reported on aldose reductase inhibiting activity of few natural products such as root of *Salacia oblonga, Salviae multiorrhizae, Glycerrhiza uralensis, Radix astragali* and puerarin. Some other studies that have also reported on aldose reductase inhibition capacities of plant products are Ajani *et al*,[47] Jung *et al.*,[48] Patel *et al.*[15,49] Reports from these studies strengthen our study because we have reported in this study that *L. cyanescens* also contains phenol, flavonoid and other phytochemicals. We have also reported in this study that the $IC_{50}$ of fractions of *L. cyanescens* ranged from 0.06 (aqueous fraction) to 1.17 mg/ml (hexane fraction).

The detection of phenols and flavonoids as reported in this study suggest that the aldose reductase inhibitory action of *L. cyanescens* may be due to these phytoconstituents. Phenolic compounds are one of the most widely occurring groups of phytochemicals and are of considerable physiological and morphological importance in plants.[41,50] Flavonoids another anti-oxidant detected in the leaves of *L. cyanescens* are commonly ingested from fruits and vegetables in the diet, although they have no nutritive value, they are capable of exerting various pharmacological activities, including antioxidative, superoxide- scavenging, and aldose reductase

inhibitory activity.[49] Several flavonoids, such as quercitrin, guaijaverin and desmanthin have been tested and proven for their inhibitory activity against aldose reductase.[51] A previous study by Mutiu and Theophilus[52] reported that ethanol leaf extract of *L. cyanescens* possessed hypoglycaemic effect, and that one of the mechanisms by which this extract elicited its hypoglycaemic property may be through the inhibition of diabetes-related enzymes (pancreatic *α*-amylase and intestinal *α*-glucosidase) activities. In the present study, we also reported that the leaves extract of *L. cyanescens* may be effective in preventing the secondary diabetic complication particularly cataract, and that this effect may be due to the ability of the plant to inhibit aldose reductase activity.

In order to elucidate the mode of inhibition of aldose reductase enzyme by *L. cyanescens*, the kinetic study was performed for the entire fraction. Effect of different fractions on rat lens aldose reductase activity in Lineweaver-Burk plot using DL glyceraldehyde as a substrate was made between 1/velocity vs 1/DL-glyceraldehyde. Data obtained in the study suggest that the aldose reductase inhibitory compounds present in *L. cyanescens* leaves extract/fractions can interact and inhibit lens aldose reductase enzyme. Kinetic study performed to understand the type of inhibition suggest that these compounds can interact and inhibit AR enzyme in both competitive and un-competitve manner. At the macroscopic level, the effect of competitive inhibition is to increase the substrate concentration required to achieve a given reaction velocity; in other words, to raise the $K_m$. The $V_{max}$ is unchanged, however. Mixed inhibition is a type of reversible inhibition which combines the effects of both competitive and uncompetitive inhibition. The inhibitor can bind either the enzyme or the enzyme-substrate complex and in either case will form an inactive complex. The inhibitor does not bind to the substrate binding site and therefore is not a substrate analogue. The value of $V_{max}$ will go down; the Km can go up or down.[53] The data obtained suggest that aqueous fraction had significant activity against aldose reductase enzyme, this may be due to the presence of the phenol and flavonoid contents. A lower $K_m$ corresponds to a higher affinity. The presence of an uncompetitive inhibitor actually increases the affinity of the enzyme for the substrate. Since the inhibitor binds the E-S complex, the inhibitor decreases the concentration of the E-S.[53] Despite their rarity in drug discovery programs, uncompetitive inhibitors could have dramatic physiological consequences. As the inhibitor decreases the enzyme activity, there is an increase in the local concentration of substrate. Without a mechanism to clear the buildup of substrate, the potency of the uncompetitive inhibitor will increase.

Reactive oxygen species (ROSs) are known to damage cellular membranes by inducing lipid peroxidation. They also can damage DNA, proteins, lipids and chlorophyll.[54] The most popular ROS are $\cdot O_2^-$ (superoxide radical), $H_2O_2$ (hydrogen peroxide) and $\cdot$OH (hydroxyl radical) originating from one, two or three electron transfers to dioxygen ($O_2$). Under physiological conditions $\cdot O_2^-$ is not very reactive against the biomolecules of the cell and in aqueous solutions at neutral or slightly acidic pH disproportionates to $H_2O_2$ and $O_2$. $H_2O_2$ is relatively stable and not very reactive, electrically neutral ROS, but is very dangerous because it can pass through cellular membranes and reaches cell compartments far from the site of its formation.[11] Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases. Cataract formation has been attributed to oxidative stress triggered by these reactive oxygen species (ROS). All the fractions investigated in this study showed free radical scavenging activity. Data from the study further revealed that the aqueous fraction has a greater reducing power and was more efficacious than all other fractions in scavenging free radicals and also as a metal chelator. Plants produce a very diverse group of secondary metabolites with antioxidant potential. A report by Catherine *et al.*[55] indicated that Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C many dietary polypenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamin E and C and thus might contribute significantly to protective effect *in vivo*. studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and thus might contribute significantly to the protective effects *in vivo.* The antioxidant activity of polyphenols has been attributed to their ability to scavenge free radicals (which allow them to act as reducing agents and hydrogen or electron donor) or chelate metal cations.[41,50,56] Zhang *et al.*,[57] reported that the presence of electron-donating and electron-withdrawing substituents in the ring structure of phenolics as well as the number and arrangement of the hydroxyl groups determines their antioxidant potential. Our result alsosuggests that the free radical scavenging capacities of the antioxidants present in *L. cyanescens* is correlated with the solvent employed in the fractionation. This therefore indicates that the antioxidant potential of compounds varies with the polarities of the solvents. This observation agrees with the report of Moure *et al.*,[58] Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants.[59]

## CONCLUSION

Result of this study indicates that the leaves of *L. cyanescens* has a strong aldose reductase and free radical scavenging activity against DPPH, $H_2O_2$, NO, $O_2^-$, OH. These activities, the study revealed is more concentrated in the aqueous fraction indicating that the efficacy of *L. cyanescens* extract in inhibiting aldose reductase enzyme is dependent on extractive solvents. In order to effectively utilized the plant as a base for anticataractogenin agent, there is need to further isolate and characterize the antioxidant constituents responsible for this action and to confirm these observed results in *in vivo* studies. It is also important to determine the toxicological implication of the plant and to identify the bioactive compound responsible for these observed actions. This is the focus of on going study in our laboratory.

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The authors declare that there are no conflicts of interest.

## CONFLICT OF INTEREST

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