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# Aldose reductase inhibitory potential of different fractions of *Houttuynia cordata* Thunb

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ABSTRACT

**Objective:** To evaluate the aldose reductase (AR) inhibitory activity of different fractions from *Houttuynia cordata* (*H. cordata*) which used as a medicinal salad for lowering of blood sugar level. **Methods:** AR inhibitory activity along with protein content was evaluated *in vitro* in rat lens. Total phenol and flavonoid contents were also determined in all the fractions. **Results:** All the four fractions were found to inhibit lens AR activity, but to different extent. From dose response curve (DRC), aqueous fraction (AQ) was found to be the most effective AR inhibitor followed by ethyl acetate (EA), chloroform (CL) and hexane fraction (HEX). The $IC_{50}$ values of AQ, EA, CL and HEX were calculated to be (64.62±3.90), (90.69±7.50), (134.59±4.90) and (151.58±3.30) μg/mL respectively. Quercetin was taken as positive control which exhibited AR inhibition with an $IC_{50}$ value of (3.21±0.60) μg/mL in a non-competitive manner. **Conclusion:** These findings indicated that, AQ fraction of *H. cordata* exhibited significant inhibitory effect on AR in a non-competitive manner, which may be attributed to the presence of high phenolic and flavonoid contents. Thus, the plant *H. cordata* may act as a promising source in the treatment of secondary complications like cataract associated with diabetes.

## 1. Introduction

Aldose reductase (AR) is a member of the aldo-keto reductase super family that reduces excess D-glucose into D-sorbitol with concomitant conversion of NADPH into NADP+[1]. This enzyme has been demonstrated to play important roles, not only in the cataract formation in the lens but also in the pathogenesis of diabetic complications that results in functional alterations of cornea, lens, retina, iris, peripheral nerve, and kidney[2]. In diabetic condition, AR increases the polyol pathway activity, which leads to an accumulation of polyol in lens fibers causing influx of water and generation of osmotic stress which finally leads to sugar cataracts[3]. The role of AR inhibitors has been extensively investigated in such complications. Literature have revealed that, cataract progression can be slowed or prevented by the use of natural therapies, particularly with plants having high flavonoid content and have shown considerable hypoglycemic and *in vivo* AR inhibitory activity[4]. Recently, plants such as *Ocimum sanctum* (*O. sanctum*) Linn, *Curcuma longa* (*C. longa*) Linn, *Azadirachta indica* (*A. indica*) (*A. Juss*), *Withania somnifera* (*W. somnifera*) L. Duanl, *Hybanthus enneaspermus* (*H. enneaspermus*) Linn F. Muell, *Ceasalpinia digyana* (*C. digyana*) Rottlerand, *Alagium lamarckii* (*A. lamarckii*) Thwaits have been reported for their AR inhibitory and anticataract potential[5-7].

The herb *Houttuynia cordata* (*H. cordata*) Thunb is a single species of its genus and is native to Japan, South-East Asia, and Himalayas. In the Ri-Bhoi district of Meghalaya, India, whole plant of *H. cordata* is eaten raw as a medicinal salad for lowering the blood sugar level and is commonly known by the name Jamyr-doh[8]. In southern China, green leaves and young roots are used as vegetable, while dried leaves are used to prepare drinks by boiling decoction. Major class of phytoconstituents reported in *H. cordata* are phenols, flavonoids and polysaccharides, whereas pharmacological activities of this plant includes hypoglycemic[9], antileukemic[10], anticancer[11],

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adjuvanticity[12], antioxidant[13]and inhibitory effects on anaphylactic reaction and mast cell activation[14]. However, there is no scientific data available for its AR inhibitory activity. Therefore, present study was aimed to evaluate the protective effects of different fractions of *H. cordata* on diabetic complications such as aldose reductase inhibitory activity in rat lens.

## 2. Material and methods

### 2.1. Chemicals used

DL-glyceraldehyde and quercetin used for AR activity determination were obtained from Sigma-Aldrich Chemical Co. (St. Louis, USA). Nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Hi-Media laboratory Pvt. Limited, India. Other reagents and solvents were of analytical grade, Double beam UV spectrophotometer (Shimadzu, Pharmaspec 1700) was used for determining the absorbance of the sample.

### 2.2. Animals

Albino rats of Charles foster strain with body weights of (160-200 g) were obtained from the Central Animal House (Reg. No. 542/02/ab/CPCSEA), Institute of Medical Science (IMS), Banaras Hindu University (BHU), Varanasi, India. Before and during the experiment, rats were fed with normal laboratory pellet diet (Hindustan lever Ltd., India) and water *ad libitum*. After randomization into various groups, the rats were allowed to acclimatize for a period of 2-3 day in the new environment before initiation of experiment. The experimental protocol has been approved by the institutional animal ethical committee (Reference no. Dean/10-11/58 dated 07.03.2011).

### 2.3. Plant material

*H. cordata* herb was collected from Jaintias Hills of Meghalaya, India. The plant was identified by Dr. B.K. Sinha, Botanical Survey of India. A voucher specimen (COG/HC/011) of the plant has been deposited in the Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi (U.P), India.

### 2.4. Preparation of extract and its fractions

Whole plant of *H. cordata* was washed with water, shade dried, ground in a mill and was passed through sieve #40 to obtain a homogenous plant powder. Dried powdered material (1 kg) of whole plant of *H. cordata* was extracted with 3 L ethanol by soxhletion until the whole plant material was completely exhausted. The resulting extract was concentrated under reduced pressure to obtain a dark crude residue (yield: 13.2% w/w). The extract was then mixed with silica gel (1:3) and was loaded in a column. Further, column was run with different solvents to obtain different fractions such as hexane: 5.3% w/w, chloroform: 3.9% w/w, ethyl acetate: 2.2% w/w and aqueous: 6.1% w/w respectively which were further dried. The fractions in a solid powdered form was then stored in a desiccator until use.

### 2.5. Determination of total phenolics and total flavonoid

Total phenolic compounds in the extracts were estimated by the Folin-Ciocalteu method using gallic acid as standard and expressed as mg/g of gallic acid equivalents (GAE)[15]. Total flavonoid content of fractions was measured as mg/g of rutin equivalents (RE) using a modified colorimetric method described previously by Kumaran and Karunakaran[16].

### 2.6. Preparation of lens homogenate

Non-cataractous transparent lenses were dissected through posterior approach from rat eye and 10% homogenate was prepared from rat lenses in 0.1 M phosphate buffer saline (pH 7.4). The homogenate was centrifuged at 5 000×g for 10 min in cooling centrifuge to obtain the supernatant. After centrifugation, the supernatant was collected and kept in ice for the determination of both AR activity and protein content. The protein content in the supernatant of the lens homogenate was determined as per the method of Lowry *et al*[17].

### 2.7. Assay of reductase activity

AR activity was assayed as per the method described by Hayman and Kinoshita[18]. A sample cuvette containing 0.7 mL of phosphate buffer (0.067 M), 0.1 mL of NADPH ($25 \times 10^{-5}$ M), 0.1 mL of lens supernatant, 0.1 mL of DL-glyceraldehyde (substrate) ($5 \times 10^{-4}$ M) to a final volume of 1 mL was read against a reference cuvette containing all components except the substrate. The enzymatic reaction was initiated after the addition of substrate. Finally, the absorbance (OD) was recorded in a double beam UV-spectrophotometer at 340 nm for 3 min at 30 s interval. AR activity was expressed as OD/min/mg protein[19]. For determination of the AR inhibitory activity of *H. cordata*, various concentrations of different fraction of *H. cordata* ranging from 25-300 μg/mL were prepared. The reaction was initiated by the addition of 0.1 mL DL-glyceraldehyde with 0.1 mL plant extract and rate of reaction was measured as described above. Different concentration of quercetin ranging from 1.25-10.00 μg/mL was prepared and was used as a standard. OD/min/mg protein was calculated for each sample. Percent inhibition of AR activity was calculated with reference to the activity of normal rat lens as 100%. The concentration of inhibitors exhibiting 50% inhibition of enzyme activity ($IC_{50}$) was calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity.

### 2.8. Kinetics of AR inhibition

The inhibition was measured in presence of different fractions of *H. cordata* and with varying concentrations of glyceraldehydes ($5\times10^{-4}$ to $30\times10^{-4}$ M). Mode of inhibition was determined by following Lineweaver-Burk plot analysis of the data and calculated according to Michaelis-Menten kinetics in order to understand the probable mode of action. $K_m$ and $V_{max}$ were also estimated. $K_i$ value in presence of fractions was determined by applying Cheg-Prusoff equation[20].

### 2.9. Statistical analysis

The data were analyzed with GraphPad Prism version 5 (San Diego, CA). Statistical analysis was done by One way ANOVA, followed by Tukey's multiple comparison test. Data are expressed as mean ± S.E.M. A level of $P < 0.05$ was accepted as statistically significant.

## 3. Results

### 3.1. Total phenolic and total flavonoid content

**Table 1.**
Phytochemical analysis and aldose reductase inhibitory activity of different fractions of *H. cordata*.

| Fractions | Yield (% w/w) | Total phenol (mg/g GAE) | Total flavonoid (mg/g RE) | ARIs activity $IC_{50}$ ($\mu$g/mL) |
|---|---|---|---|---|
| Hexane | 5.3 | 46.53 ± 0.56 | 9.33 ± 0.93 | 151.58 ± 3.3 |
| Chloroform | 3.9 | 62.91 ± 0.31[a] | 11.81 ± 1.19 | 134.59 ± 4.9 |
| Ethyl acetate | 2.2 | 83.21 ± 0.83[ab] | 24.03 ± 1.81[ab] | 90.69 ± 7.5[ab] |
| Aqueous | 6.1 | 104.01 ± 0.62[abc] | 31.62 ± 1.24[abc] | 64.62 ± 3.9[abc] |
| Quercetin | NA | NA | NA | 3.21 ± 0.6[abcd] |

Results are expressed as Mean± SEM. ($n$ = 3), [a]$P$<0.05 compared to Hexane fraction: [b]$P$<0.05 compared to Chloroform fraction; [c]$P$<0.05 compared to Ethyl acetate fraction; [d]$P$<0.05 compared to Aqueous fraction.
GAE: gallic acid equivalent, RE: rutin equivalent, NA: not analyzed

According to the data presented in Table 1, a significant difference ($P$<0.05) in the amount of the total phenolics and total flavonoids were observed among all these fractions, except total flavonoids in hexane and chloroform fraction. The richest amount of total phenolics was found to be in aqueous fraction (AQ, 36.79 mg/g GAE), followed by ethyl acetate fraction (EA, 24.41 mg/g GAE), chloroform fraction (CE, 12.02 mg/g GAE) and hexane fraction (HEX, 5.95 mg/g GAE). Similarly, the highest flavonoid content (26.23 mg/g RE) was observed in AQ, whereas HEX showed the lowest content (5.19 mg/g RE).

### 3.2. Aldose reductase inhibitory activity

The AR activity in normal rat lens was found to be (0.013 5 ± 0.005 0) $\mu$g/mL. Different fractions of *H. cordata* were found to inhibit lens AR to various extent with $IC_{50}$ values ranging from 50 $\mu$g/mL to >150 $\mu$g/mL (Table 1 & Figure 1). It is evident from the dose response curve (DRC) that AQ fraction possessed maximum AR inhibitory activity followed by EA, CH and HEX. At a concentration of 200 $\mu$g/mL, AQ fraction showed AR inhibition up to 99.97%. It is evident from the DRC that, the maximum inhibitory effects of AQ, EA, CL and HEX fractions were produced at the concentration of 200, 300, 300 and 300 $\mu$g/mL, respectively, and $IC_{50}$ values calculated was (64.62 ± 3.90), (90.69 ± 7.50), (134.59 ± 4.90) and (151.58 ± 3.30) $\mu$g/mL, respectively. Quercetin, which is well known for its potent AR inhibition, was used as a positive control which showed excellent activity with $IC_{50}$ value (3.21± 0.60) $\mu$g/mL.

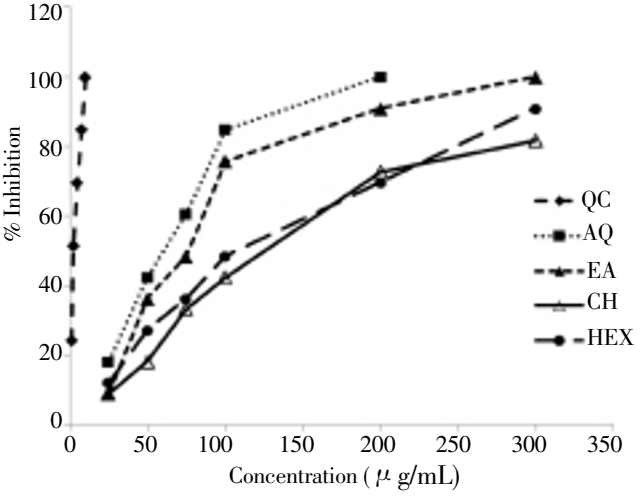


**Figure 1.** Effect of different fractions of *H. cordata* and quercetin on AR activity.

To determine the possible type of inhibition by different fractions of *H. cordata* and quercetin, a kinetic study was conducted using dl-glyceraldehyde as a substrate (concentration: $5\times10^{-4}$ to $30\times10^{-4}$ M) at 5 different concentrations for each fraction. The Lineweaver-Burk plots of 1/velocity and 1/concentration for fractions and quercetin

**Table 2.**
Enzyme kinetics of aldose reductase inhibition.

| Group | AR activity | $V_{max}$ | $K_m$ | $K_i$ |
|---|---|---|---|---|
| 1 | DL-glyceraldehyde | 0.189 00±0.001 70 | 0.000 98 × $10^{-3}$ mM | 0 |
| 2 | DL-glyceraldehyde + quercetin | 0.080 70±0.017 00[a] | 0.000 98 × $10^{-3}$ mM | 0.006 23±0.000 07 |
| 3 | DL-glyceraldehyde + aqueous | 0.098 00±0.001 10[ab] | 0.000 98 × $10^{-3}$ mM | 0.126 68±0.001 50[ab] |
| 4 | DL-glyceraldehyde + ethyl acetate | 0.139 00±0.000 50[abc] | 0.000 76 × $10^{-3}$ mM | 0.177 21±0.002 10[abc] |
| 5 | DL-glyceraldehyde + chloroform | 0.190 00±0.000 57[bcd] | 0.001 54 × $10^{-3}$ mM | 0.263 08±0.003 20[abcd] |
| 6 | DL-glyceraldehyde + hexane | 0.184 00±0.000 58[bcd] | 0.002 02 × $10^{-3}$ mM | 0.296 22±0.003 60[abcde] |

Results are expressed as Mean± SEM ($n$ = 3), [a]$P$<0.05 compared to DL-glyceraldehyde: [b]$P$<0.05 compared to DL-glyceraldehyde + quercetin; [c]$P$<0.05 compared to DL-glyceraldehyde + aqueous; [d]$P$<0.05 compared to DL-glyceraldehyde + ethyl acetate; [e]$P$ compared to DL-glyceraldehyde + chloroform.

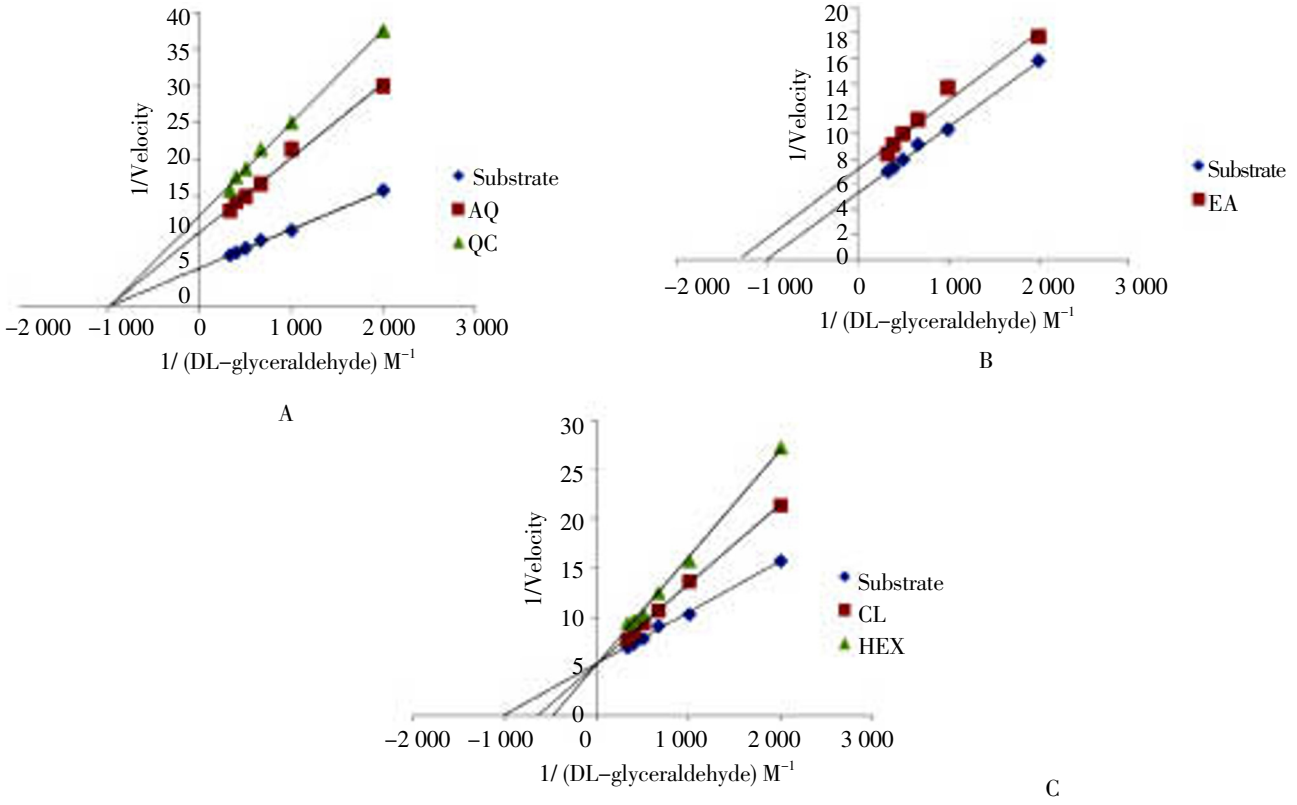


**Figure 2.** Effect of different fractions (A: aqueous fraction; B: ethyl acetate fraction; C: chloroform and hexane fraction) of *H. cordata* on the Lineweaver-Burk plot of AR activity with DL-glyceraldehyde as a substrate.

are shown in Figure 2a, 2b, 2c. The inhibitors concentration was always kept close to one which corresponds to 50% inhibition of the enzyme activity ($IC_{50}$). Data obtained from LB plot equation clearly indicated a non-competitive inhibition of AR by quercetin and AQ fraction, since they did not alter the $K_m$ but altered the $V_{max}$. Similarly, data showed a competitive inhibition of AR by CL and HEX fractions, since their $K_m$ values were altered but the $V_{max}$ remained constant. Moreover, the EA fraction showed a mixed type of inhibition (un-competitive).

Statistical analysis of the data obtained from the kinetic studies has showed significant difference between kinetic data obtained when DL-glyceraldehyde alone and DL-glyceraldehyde along with fractions of *H. cordata* ($P<0.001$). A Low $K_m$ value suggests that AR has higher affinity towards substrate (DL-glyceraldehyde). Statistical analysis by one-way ANOVA revealed that, there was a significant difference between the $V_{max}$ and $K_i$ values which showed that, AQ fraction offers highest inhibition towards enzyme.

## 4. Discussion

Blindness in diabetics is largely due to retinopathy and/or cataract. Cataract is the opacification of the lens, which interferes with the transmission of light on to the retina. Cataract alone is responsible for the cause of around 50% blindness worldwide. Activation of polyol pathway due to increased activity of aldose reductase has been implicated in diabetic cataract. This is a two step metabolic pathway in which glucose is reduced to sorbitol, which is then converted to fructose. AR (alditol: NADP+ 1-oxidoreductase, EC 1.1.1.21) has been implicated in the etiology of complications of diabetes such as neuropathy, nephropathy and retinopathy including catatractogenesis. It has been reported that, the cataract progression can be slowed or prevented by the use of natural therapies, particularly with plants having high flavonoid content mainly by AR inhibitory effect[21].

Quercetin, is a potent AR inhibitor for sorbitol accumulation in polyol pathway, therefore it was used as a standard in our study. The mode of inhibition of different fraction of *H. cordata* and quercetin indicated a non-competitive inhibition of AR by quercetin as well as AQ fraction, since they did not alter the $K_m$ but altered the $V_{max}$. Low range of $IC_{50}$ value by AQ fraction to that of quercetin suggests the effectiveness AQ in inhibiting AR. The non-competitive inhibitors, in general, are those substances that form strong covalent bonds with enzyme and consequently are not displaced by the addition of excess substrate and hence form irreversible reactions. Since, the richest amount of total flavonoids and phenols was found in the AQ fraction, they may render irreversible inhibition of AR by successfully blocking the polyol pathway that leads to catractogenesis.

The plant *H. cordata* has been selected for the RLAR inhibitory activity as it was found to be an important source of natural polysaccharides, phenols and flavonoids[21-23]. Moreover, the hydroalcoholic extract has been shown to possess significant antioxidant and hypoglycaemic activity.

The results of our study suggested AR inhibitory activity of entire fractions with different magnitude. AQ fraction was found to be most potent inhibitor of AR followed by EA, CL and HEX fractions. Looking at the AR inhibitory potential, it is suggested that the above plant could be a potential tool in treatment of diabetic retinopathy which may circumvent the toxic effects of clinically tested AR inhibitors such as sorbinil, statil, epalrestat, tolrestat and alrestatin.

In the present study, AR inhibitory activity of different fractions of *H. cordata* was carried out on rat lens aldose reductase enzyme. Among the tested fractions, aqueous fraction showed potent AR inhibition activity, which may be attributed to the presence of high water soluble flavonoid contents. The mode of inhibition of AQ was similar as that of the positive control quercetin. These results suggest that, *H. cordata* could potentially provide a new natural treatment for diabetic complications. However, further in vivo studies on the effects of these flavonoids on sorbitol accumulation is required to treat cataract.

## Conflict of interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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