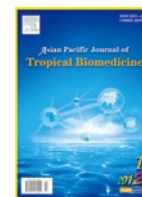




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Aldose reductase inhibitory activity of alcoholic extract of *Pedaliium murex* Linn fruit

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ABSTRACT

Objective: To investigate physico–chemical investigation and aldose reductase inhibitory activity in rat lens aldose reductase (AR) enzyme of the ethanolic extract of fruits of *Pedaliium murex* (*P. murex*) Linn (Pedaleaceae). **Methods:** Physico–chemical investigation of ethanolic extract of fruit of the *P. murex* (EPM) was carried out. EPM was screened for aldose reductase inhibitory activity using rat lens AR enzyme. HPTLC fingerprinting analysis of EPM was performed in chloroform: methanol (8:2) solvent system. **Results:** Total ash, acid insoluble ash, water soluble ash, alcohol and water soluble extractive value and loss on drying of *P. murex* were found to be 4.2%, 1.2%, 0.92%, 6.2%, 5.0% and 7.5% respectively. Eleven prominent spots were found to be present in the EPM in HPTLC fingerprint analysis. EPM showed significant level of aldose reductase inhibitory potential [IC₅₀ value (57.20±3.68) μg/mL]. From the value of V_{max}, K_m and K_i, it was found that EPM exhibits non–competitive inhibition with AR enzyme. **Conclusion:** The present study reveals scientific basis of the *P. murex*, which can be used in the treatment of the cataract disorder.

1. Introduction

Adequate amount of antioxidant is necessary in the body to prevent body from oxidative stress, which causes various type of complication such as atherosclerosis, carcinogenesis, inflammation, neurodegenerative, cardiovascular and kidney disease. When excess production of the reactive oxygen species (ROS) occurs in the body, it damages several molecules in the body like DNA, protein and lipids and causes various type of complication in kidney, eye, blood vessel, and nerve tissue[1,2]. Plants are good source of natural antioxidant due to the presence of the different type of chemical constituents (secondary metabolite), can prevent many types of disease by inhibiting the peroxidation chain reaction. It also protects the body from various complications and reduces the oxidative damage in the body[1,3].

Plants containing natural antioxidants (tannins, flavonoids,

vitamins C and E) can preserve β–cell function and prevent ROS formation[4]. Polyphenols, which are classified into many groups such as flavonoids, tannins, stilbenes with known health–beneficial properties possessed free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti–inflammatory action and antidiabetogenic potentiality[5].

Singlet oxygen may damage and cross–link lens proteins and oxidants play an important role in the eye disorders such as cataract and retrolental fibroplasia[6]. Studies have shown that consumption of foods and beverages containing good amount of the phenolic have low risk of oxidative stress and other related disorder[7]. Increase AR activity in the body is due to high level of glucose which results in decreased NADPH/NADP⁺ ratio. This affects the enzymes such as nitric oxide (NO) synthase and glutathione reductase (GSH) leading to oxidative stress[8].

AR inhibitors can prevent or reverse the abnormalities associated with diabetic complications. Many kinds of AR inhibitors from natural sources, such as flavonoids (quercetin, guaijaverin, and desmanthin–1) showed good inhibitory potential against aldose reductase[9]. Flavonoid which is found in citrus fruit has inhibiting power against certain enzymes such as xanthine oxidase, aldose reductase,

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and shows antioxidant activity^[10]. At present, there is fewer number of therapies available for the treatment of diabetic complications. An approach to control excess glucose level in tissue and inhibition of first step of polyol pathway by aldose reductase inhibitors is great significance^[11].

Pedaliium murex (*P. murex*) (Pedaliaceae) is very popular under the Hindi local name 'Gokhru'. It is found in different parts of the world such as tropical Africa, Srilanka, India, Mexico and Pakistan. The fruits are rich in flavonoids, saponin, sterol and soluble proteins^[12]. An infusion or extract prepared from the different parts of *P. murex* in cold water is used as demulcent, diuretic and also found to be used in the treatment of disorders of urinary systems such as gonorrhoea, dysuria, incontinence of urine and vice versa^[6,7]. The plant is also used by the local people as analgesic and antipyretic activities^[13,14].

P. murex is used mainly as tonic, aphrodisiac and useful in strangury, urinary discharges, vesicular calculi, cough, asthma, pain, skin diseases, heart troubles, piles, leprosy, stomachic, headache, diarrhoea, dysentery, cough and intestinal affections^[15,16]. Pharmacologically, the plant have been investigated for antiulcerogenic, nephroprotective, hypolipidemic, aphrodisiac, antimicrobial and insecticidal activities^[17–20]. Due to its significant use in traditional medicine it is worthwhile necessary to standardize the fruits of *P. murex* and to investigate its aldose reductase inhibitory potential.

2. Material and methods

2.1. Chemicals and instruments

DL-glyceraldehyde, NADPH, quercetin and rutin were obtained from Sigma Aldrich. All other chemicals and solvents used were of analytical grade. The absorbances were recorded using the ultraviolet–visible spectrophotometer of (Shimadzu, Pharmaspec–700).

2.2. Plant materials collection and extraction

The fruits of *P. murex* was purchased from the herbal supplier in Varanasi and authenticated by Professor S. D. Dubey, Department of Dravyaguna, Institute of Medical Sciences; Banaras Hindu University, Varanasi, and specimen sample (COG/PM/01/08) was kept in Department of Pharmaceutics IT–BHU, Varanasi for further reference. 1 kg ground powder was extracted in ethanol by hot extraction technique. The solvent was filtered and concentrated in vacuum in a rota evaporator to obtain the dry extract. The dried extract was kept in desiccator until tested.

2.3. Quality control standardization

Physico–chemical analysis *i.e.* percentage of ash values,

loss on drying, extractive values and fluorescence analysis were performed according to the official procedure^[21,22]. HPTLC fingerprinting analysis (CAMAG) of ethanol extract was performed in chloroform: methanol (8:2) solvent system, mode of application was automatic, volume of sample was 5 μ L, development mode was ascending, and scanning was done at 254 nm by use of Wincats software.

2.4. Aldose reductase inhibitory activity

Rat lens were used as an AR enzyme sources for the determination of AR inhibitory activity, where as for kinetic study Lineweaver–Burk plot was used.

2.4.1. Experimental animals

Male Charles foster rats (180–250 g) were obtained from central animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals received standard pellet diet (Mona laboratory animal feed) and water ad libitum and were maintained under standard environmental conditions [12 h light/12 h darkness, (21 \pm 2) $^{\circ}$ C].

The experimental study was approved by the Institutional Animal Ethical Committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

2.4.2. Purification of lens AR

Eyes of normal Charles foster rats removed under sterile condition were separated from posterior approach, washed with saline and kept in ice. 10% homogenate was prepared from rat lenses in 0.1 M phosphate buffer saline (pH 7.4), centrifuged at 5 000 \times *g* for 10 min in a cooling centrifuge to get the supernatant. Total protein content of the supernatant was determined according to the method described by Lowry *et al* (1951)^[23,24].

2.4.3. Enzyme assay

For the determination of the Lens AR activity in a sample cuvette 0.7 mL of phosphate buffer (0.067 M), 0.1 mL of NADPH (25×10^{-5} M), 0.1 mL of lens supernatant, 0.1 mL of DL-glyceraldehyde (substrate) (5×10^{-4} M) were taken, absorbance of the final solution was taken against a reference cuvette containing all components but the substrate, DL-glyceraldehyde.

The enzymatic reaction was started by the addition of the substrate and the absorbance (OD) was recorded at 340 nm for 3 min at 30 s interval. AR activity was expressed as OD/min/mg protein. For determination of the AR inhibiting activity of plant extract, various stock solutions of the EPM were added to both the reference and standard cuvettes. The reaction was initiated by the addition of 0.1 mL DL-glyceraldehyde and the rate of reaction was measured as described above^[23,24].

2.4.4. Enzyme kinetics

For study of enzyme kinetics value of K_m , K_i and V_{max} of AR were determined using DL-glyceraldehyde as substrate in the absence and presence of different concentrations of EPM by Lineweaver–Burk double reciprocal plots^[23,24].

2.5. Statistical analysis

Results are expressed as mean value \pm standard error mean (SEM) of three independent determinations. Linear regression analysis was performed, quoting the correlation coefficient r^2 . Two-ways ANOVA followed by Bonferroni post test was performed for evaluation of all data. GraphPad Prism (Version 5) software was used for all statistical analysis, and $P < 0.05$ was considered as significance.

3. Results

3.1. Quality control standardization

The physico-chemical constants viz. percentage yield, total ash, acid insoluble ash, water soluble ash, alcohol soluble

extractive, Water soluble extractive and loss on drying were found to be 3.6%, 4.2%, 1.2%, 0.92, 6.2%, 5.0% and 7.5%.

Florescence study was performed by reacting EPM with different chemical reagents and monitored both in day light as well as in UV light, which was represented in Table 1. Further the fingerprinting analysis by HPTLC technique showed that EPM contain eleven spot in which three spots were found to be prominent (Figure 2).

3.2. Aldose reductase inhibitory potential

In the present study the evaluation of AR inhibitory activity was found to be significant in inhibiting the rat lens AR enzyme to various extents ranging from 0.1 mg/mL to >1 mg/mL. The AR activity of normal rat lens was found to be 0.0144 ± 0.0007 , which was considered as a 100% inhibition. EPM showed significance percentage of inhibition [IC_{50} value (57.20 ± 3.68) μ g/mL] but lesser as compared to the standard quercetin [IC_{50} value (2.83 ± 0.15) μ g/mL]. The AR inhibitory activities of EPM are presented in the Figure 1, where quercetin was used as a standard.

Table 1

Fluorescence analysis of the *P. murex* fruit powder.

S. No.	Mixture + Powder	Fluorescence in day light	Fluorescence in U.V. light
1	Powder as such	Brown	Light brown
2	Powder + 1 N NaOH in methanol	Golden rod	antiquate
3	Powder + 1 N NaOH in water	Peru	Lime green
4	Powder + 1 N HCl in methanol	Yellow	Pale green
5	Powder + 1 N HCl in water	Cornsilk	Light green
6	Powder + 1 N HNO ₃ in methanol	Golden rod	Green yellow
7	Powder + 1 N HNO ₃ in water	antiquate	Medium see green
8	Powder + 5% Iodine	Orange	Light green
9	Powder + 5% FeCl ₃	Peru	Not Fluorescence
10	Powder + 50% KOH	Brown	Green yellow
11	Powder + NH ₃	Peru	Medium sea green
12	Powder + Picric acid	Yellow	Not Fluorescence
13	Powder + Acetic acid	Yellow	Lime green

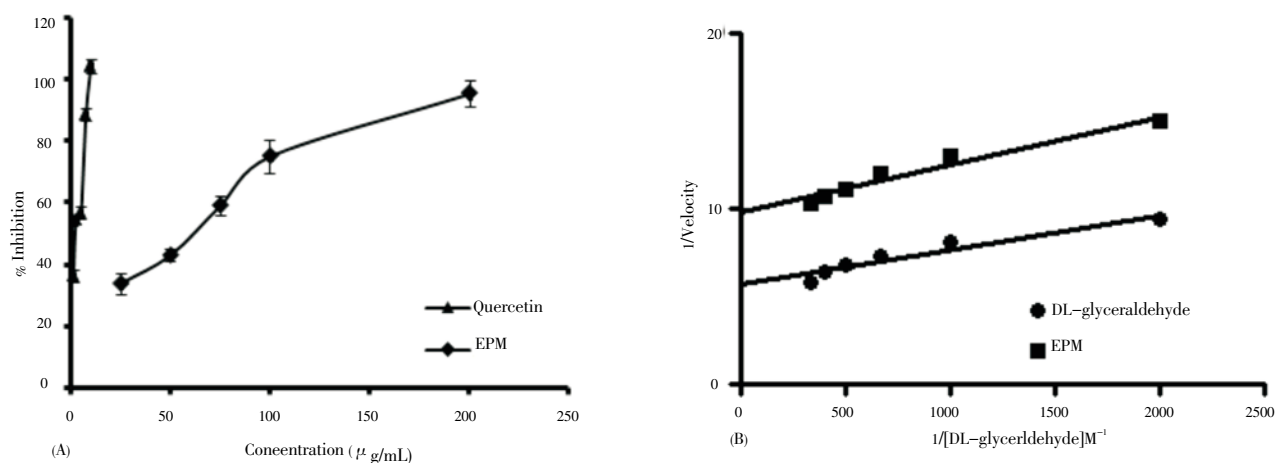
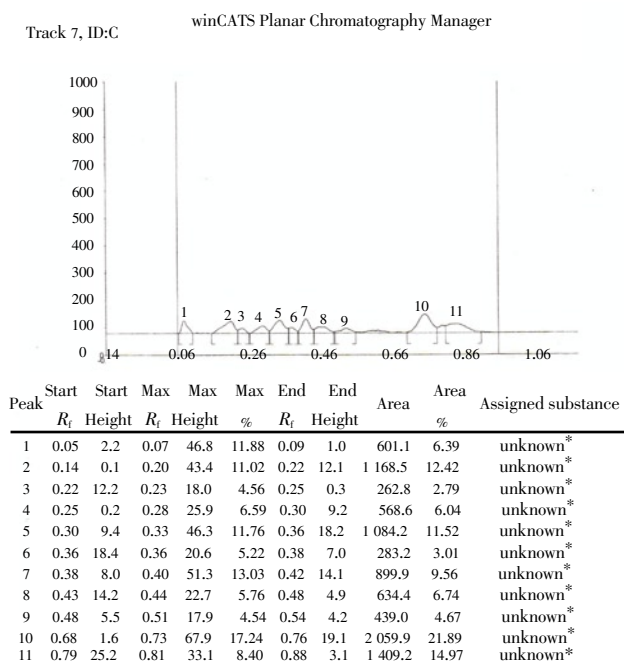


Figure 1

(A): Effect of EPM and quercetin on aldose reductase enzyme (B): Effect of EPM on the Lineweaver–Burk plot of AR activity with DL-glyceraldehyde as substrate.

3.3. Kinetic study

Study of enzyme kinetics was performed for EPM to understand the mechanism involved in the inhibition process. It was found that EPM showed non-competitive inhibition as V_{\max} [0.1853 ± 0.0006], K_m [0.4402 ± 0.0004] $\times 10^{-3}$ mM] and K_i [0.0606 ± 0.0016] $\times 10^{-3}$ mM] did not differ as compared to the substrate DL-glyceraldehyde [0.1378 ± 0.0168], (0.6089 ± 0.0012) $\times 10^{-3}$ mM]. The Lineweaver–Burk plot for EPM was presented in the Figure 1.



4. Discussion

Ethnopharmacological data shows that *P. murex* have significance pharmacological activity, based upon the data the present study was to mainly focus on the protective effects of EPM on AR enzyme system. Quality control standardization was also carried out to obtain the proper authenticity of the plant and to check the genuine nature of this plant from the adulterated ones.

The physico-chemical parameter like percentage of ash values, loss on drying and extractive values reported in this paper can be used as an analytical tool for the qualitative standardization of *P. murex* fruit. For analysis of the powdered drug physical and chemical parameters is some time inadequate, then plant material can be identified from their adulterants on basis of fluorescence study.

HPTLC analysis showed eleven components in which two are densely prominent, so in future the isolation of these two components can be used as a marker compound for the standardization of the *P. murex* plant material. Alcohol was used as a solvent for extraction

due to its high extractability and non toxicity.

Aldose reductase is the rate limiting enzyme which reduces glucose to sorbitol in polyol pathway. Production of the excess of sorbitol in the body is the main factor responsible for the development of secondary complication such as cataract. So inhibition of AR enzyme in the body might be beneficial in the early onset of complication, which occurs due to the excess accumulation of the sorbitol in the body such as diabetes cataract.

From the present study it was found that EPM showed significant inhibitory potential against AR enzyme so it can be used to treat complication such as diabetic cataract. From the earlier work it was reported that some flavonoids, polyphenols and sugar derivatives have inhibitory potential against α -glucosidase and AR enzyme[25].

Plant such as *Salacia oblonga* and *Salviae multiorrhizae*, *Glycerrhiza uralensis*, *Radix astragali* have significant amount of bioflavonoids, which have been reported to have AR inhibitory activity[24]. In another study it was found that active constituent such as quercetin, quercitrin, flavonol and flavanone having the 7-hydroxy and catechol moiety at the B ring exhibit the strong activity against AR enzyme[26].

Type 2 diabetes patients face higher levels of oxidative stress mainly due to depletion of majority of the plasma antioxidants, which causes lipid peroxidation, DNA damage, and enzyme inactivation including tissue damage. Due to significance antioxidant activity, EPM can be used to treat the imbalance of antioxidant and othe diabetic complication in the body system.

Kinetic study reveals the nature and type of inhibition, based on the data obtain in the present study it was found that EPM had significant activity against AR enzyme. Various types of compound were developed for the treatment of secondary complications of diabetes such as cataract and withdrawn due to side effect suggesting that very less number of potent inhibitor against AR enzyme currently exists[11]. In Enzyme kinetic study, Lineweaver–Burk plot using DL-glyceraldehyde as substrates are used and found that EPM had non-competitive inhibition.

In conclusion, the present investigation suggests that the fruit of *P. murex* showed significant aldose reductase inhibitory activity *in vitro* may be due to the presence of the different type of constituents. However further study is necessary to know the exact mechanism of action and the compounds responsible for the AR inhibitory activity. The present study reveals the significance of EPM for the treatment of the secondary complications of diabetes mellitus such as cataract.

Conflict of interest statement

The authors report no conflict of interest

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