IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, S.Iss 07, 2022

IN VITRO ANTIOXIDANT AND ALDOSE REDUCTASE INHIBITORY ACTIVITY OF FOENICULUM VULGARE MILL.FRUIT

[1] Vandana Dongare, [2]Nilofar Naikwade, [3]Chandrakant Magadum, [4]Akalpita Arvindekar

[1,2]Appasaheb Birnale College of Pharmacy, Sangli. South Shivajinagar, Sangli-Miraj road, Sangli 416416. (M.S.) India.

[3]Rajarambapu College of Pharmacy, Kasegaon (M.S.) India.

[4]Department of Biochemistry, Shivaji University, Kolhapur (M.S.) India.

gavadepj@gmail.com nilofar_naikwade@rediffmail.com

auarvindekar@rediffmail.com

Abstract:

Objectives: The present study was to evaluate in vitro antioxidant activity and to study the aldose reductase inhibitory effect of methanolic extract of Foeniculum vulgare Mill. Fruit.

Methods: Qualitative and quantitative antioxidant activities were evaluated by free radical scavenging method using stable radical DPPH (80µg/ml). The reducing power of the extract was also determined using potassium ferricyanide and increase in absorbance was measured on UV spectrophotometer. Aldose reductase inhibitory activity was determined by determining the decrease in NADPH conc. at 340nm using UV spectrophotometer. The two isolated fractions of methanolic extract obtained by column chromatography were screened for antioxidant and aldose reductase inhibitory activity.

Results: Qualitative antioxidant activity of methanolic extract showed white spot against pink background it indicates presence of antioxidant activity. The extract showed higher reducing power activity. The methanolic fraction showed higher antioxidant and aldose reductase inhibitory activity than chloroform fraction. In reducing power assay there is increased absorbance of the reaction mixture indicates stronger reducing power.

Conclusion: The results concluded that the methanolic extract of Foeniculum vulgare Mill fruit having stronger antioxidant and aldose reductase inhibitory activity .

Key words: Aldose reductase, Antioxidant, Cataract, Foeniculum vulgare, Oxidative stress.

List of abbreviations
1) AR-Aldose Reductase.
2) DPPH-1, 1-diphenyl-2picrylhydrazyl.
3) TLC- Thin Layer Chromatography.

Introduction:-

Diabetes mellitus is a disorder of carbohydrate, fat and protein metabolism attributed due to diminished production of insulin or insulin resistance **[1]**. Diabetes is associated with significant oxidative stress & that oxidative damage to tissue may be a contributory factor to several diabetic complications **[2].** Increasing evidence from both experimental & clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes mellitus; free radicals are formed disproportionately in diabetes by glucose oxidation, non enzymatic glycation of proteins & subsequent oxidative degradation of glycated proteins **[3].** Antioxidants are the substances, when present in small quantities prevent the oxidation of cellular organelles by minimizing the damaging effects of oxidative stress **[4]** Recent evidence strongly suggests that the enzyme aldose reductase plays a primary role in the initiation of the cataractus process in experimental galactosemia and diabetes. **[5]** Cataractogenesis is a common complication that occurs in diabetes mellitus. Aldose reductase (AR), the key enzyme of the polyol pathway, is known to play important roles in diabetic complications. The inhibitors of aldose reductase, therefore, would be potential agents in the prevention of one of these complications, namely, the development of cataracts **[6]**.

The association between diabetes and its long term complications, such as cataracts, has been well established. Thus, drugs treating the short term complications of diabetes (i.e. uncontrolled plasma glucose levels and their immediate effects) should also be capable of preventing or delaying the development of long term complications **[7].** Thus oxidative stress is prime cause of cataract **[8]**. The most abundant types of antioxidative compounds in human diet are flavonoids **[9]**. Use of aldose reductase inhibitors and antioxidants can prevent secondary complications.

Materials and methods:-

Preparation of extract:-

Air dried & powdered Foeniculum vulgare Mill. Fruit was extracted with methanol by using soxh let extraction assembly. The extract was subjected for evaporation on steam bath at 75^0C. The thick paste of extract was obtained **[10]**.

Antioxidant assay:-

DPPH solution in methanol (80µg/ml) was used for the free radical scavenging property of the extracts as well as two isolated spots, querecitin; a natural antioxidant was used for positive control.

I) Qualitative assay:-

Test methanolic extract & std. querecitin were run on TLC using solvent system ethyl acetate: formic acid: glacial acetic acid: water (25:2.75:2.75:9).The two isolated spots were obtained on TLC for extract. TLC plate sprayed with DPPH solution using an atomizer and was allowed to

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, S.Iss 07, 2022

develop for 30 min. The white spot against pink backgrounds indicated the antioxidant activity **[11]**. This bioactive methanolic extract was fractionated over a silica gel column by elution with solvents of increasing polarity in the order of chloroform and methanol. These two fractions give positive ferric reaction for phenol and reddish pink colour on Shinoda test showing it to be a flavonoid **[12]**. Chloroform and methanol fraction were taken for the quantitative antioxidant & aldose reductase inhibitory activity.

II) Quantitative assay:-

The conc. of methanolic extract 2 mg/ml & the conc. of two isolated fractions 20µg/ml prepared in methanol. Then 1ml sample is to be tested was mixed with DPPH solution (80 µg/ml) & allowed to stand for half an hr. to occur the reaction. The experiment was performed in triplicate & the average absorption was noted. The antioxidant activity was calculated using the following formula **[9]**

$$\text{Antioxidant activity} = 100 \times \left(1 - \frac{\text{Absorbance with compound}}{\text{Absorbance of the blank}}\right)$$

III) Reducing power assay:

Principle:

The reducing power of methanolic extract of Foeniculum vulgare Mill fruit was determined by the method of Oyaizu 1986.Substances, which have reduction potential ,react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Antioxidant

Potassium ferricyanide + Ferric chloride ⟶ Potassium ferrocyanide + ferrous chloride **[13]**.

Assay of Reducing Power: This was carried out as described previously 1 ml of plant extract solution (final concentration 100-500 mg/l) was mixed with 2.5 ml phosphate buffer(0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [K3Fe(CN6)] (10g/l), then mixture was incubated at 50^0C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml Fecl3 (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power **[14]**.

In vitro aldose reductase inhibitory activity studies.

Preparation of aldose reductase:

1 gm eye lenses were pooled and homogenized in 3 volume (1:3 w/v) of 0.9% Nacl, containing 0.5mM PMSF and 10 mM β-mercaptoethanol, centrifuged at 10,000g for 30 min at 4^0 C. The supernatant was used for determination of AR activity. The activity was calculated as **15]**:

$$\text{Activity U/ml} = \frac{\Delta A \text{ test/min} - \Delta A \text{ control/min}}{6.2 \times \text{volume taken for analysis} \times \text{total volume (ml)}}$$

Where 6.2 = micro molar extinction coefficient of NADPH at 340 nm

AR activity:

AR activity of the freshly prepared enzyme was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm in Jasco V-550 spectrometer. 40mM xylose used as substrate. Assay mixture containing 0.067M buffer, 40mM xylose, 400mM LiSo4, enzyme and 0.125mM NADPH. Decrease in concentration was recorded at 340 nm for 5 min. at 37^0C) **[16]**.

AR inhibitory activity
The inhibitory activity of the methanolic extract and two isolated fractions on aldose reductase were carried out using all the optimized data in a 2 ml cuvette optimized amount of enzyme , inhibitor, 0.067M buffer(PH6.2), 0.125mM NADPH, 400mM LiSO4 and40mM xylose (substrate) were taken. Decrease in concentration was recorded at 340 nm for 5 min. at 37^0C.
Statistical Analysis: The results are presented as means ±SEM. All parameters were analyzed using Dunnet's Test. $P<0.01$ was considered significant.

Results: DPPH method was used to evaluate the free radical scavenging ability of the methanolic extract as well as the isolated fractions. The percent inhibition of the DPPH radical shown in table 1 and the qualitative antioxidant activity of the methanolic extract gives white spot against pink background indicates presence of antioxidant activity shown in fig 1. Methanolic extract and isolated fraction showed significant antioxidant activity.

Table 1 shown aldose reductase inhibitory activity of methanolic fraction is higher than that of chloroform fraction. Table 2. Shows reducing power of methanolic extract of Foeniculum vulgare as compared to Ascorbic acid.

Discussion:

Table1 shows antioxidant activity (Free radical scavenging activity) & aldose reductase inhibitory activity .Reactive oxygen species are an important part of the defence mechanism against infection, but excessive generation of free radicals on unsaturated fatty acids has been implicated in the pathogenesis of vascular disease. Diabetic patient have an increased incidence of vascular disease and it has been shown that free radical activity is elevated during diabetes **[17]**. Thus risk of diseases can be reduced by increased consumption of antioxidants which are abundant in food **[18]**. The role of antioxidant and inhibition of aldose reductase in diabetes taking in to consider, in this study we have investigated the aldose reductase inhibitory activity and antioxidant activity of Foeniculum vulgare by DPPH scavenging assay and reducing power of the extract.Fig.1 shows the methanolic extract showed white spots against pink background it indicates presence of antioxidant activity. The quantitative % antioxidant activity showed methanolic fraction has higher antioxidant activity than chloroform fraction. Fig. 4 shows increase in concentration gives increase in absorbance.

The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Comparison of the antioxidant activity of the extract and ascorbic acid is shown in Figure 2.
Table 2. Shows reducing power of methanolic extract of Foeniculum vulgare as compared to Ascorbic acid. The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductants (i.e. antioxidants) in Foeniculum vulgare extracts causes the reduction of the Fe 3+/ferricyanide complex to the ferrous form. Therefore, the Fe 2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 4 shows the reductive capabilities of the Foeniculum vulgare extracts compared to ascorbic acid. The reducing power of Foeniculum vulgare extracts was very potent and the power of the extract was increased with quantity of sample [14].

Table 1 shows the % antioxidant and % aldose reductase inhibition by the extract and isolated fractions. Oxidative stress is one of the causes of secondary complications. Accumulation of high concentrations of polyols in the lens leads to excessive hydration, sodium overload and loss of potassium ions due to an increase in intracellular ionic strength. The resulting hyperosmotic stress associated with oxidative insult is postulated to be the primary cause for the development of diabetic complications such as cataract **[19]**. Inhibition of the polyol pathway can prevent the formation of sorbitol and fructose **[20]** and thus prevent the secondary complications such as nephropathy, neuropathy, retinopathy and cataract. Fig. 3 shows methanolic fraction higher potent than chloroform fraction for aldose reductase inhibition.

Conclusion:

In diabetes activation of the polyol pathway and oxidative stress causes secondary complications. Use of natural antioxidant compounds of the plant and aldose reductase inhibitors can prevent the secondary complications. The results concluded that Foeniculum vulgare having strong antioxidant and aldose reductase inhibitory activity.

Acknowledgement:

The author is thankful to Principal Shri D. D. Chougule, Appasaheb Birnale College of Pharmacy, Sangli and Shivaji University for providing facilities.

References:-

1) Claudia E.N.MOMO, Julius E. OBEN, Dagobert TAZOO, Eticnne DONGO (2006). Antidiabetic and hypolipidemic effects of Laportea ovalifolia (Urticaceae) in alloxan induced diabetic rats. Afr. J. Traditional, Complementry and Alternative Medicines; 3(1):36-43.

2) G. Manonmani, V.Bhavapriya, S. Kalpana, S.Govindasamy, T.Apparanantham (2005). Antioxidant activity of Cassia fistula (Linn.) flowers in alloxan induced diabetic rats. Journal of Ethnopharmacology; 97:39-42.

3) A. Lukacinova, J.Mojzis, R.Benacka, J.Keller, T.Maguth, P.Kurila, et al (2008). Preventive Effects of Flavonoids on Alloxan-Induced Diabetes Mellitus in Rats. ACTAVET; 77:175-182.

4) K.Indira Priyadarsini (2005). Molecular Mechanisms Involving Free Radical Reactions of Antioxidants and Radioprotectors. Founder's Day Special Issue; 1-6.

5) J.A. Jedziniak and J.H.kinoshita (1971). Activators and inhibitors of lens aldose reductase. Investigative Ophthalmology; 10(5):357-366.

6) Dr.Angel Guzman and Dr. Ricardo O. Guerrero (2005). Inhibition of aldose reductase by herbs extracts and natural substances and their role in prevention of cataracts. Rev Cubana plant Med; 10:3-4.

7) Ester Cohen-Melamed, A.Nyska, A. Pollack and Z.Madar (1995). Aldose reductase (EC 1.1.1.21) activity and reduced glutathione content in lenses of diabetic sand rats (Psammomys obesus) fed with acarbose. British Journal of Nutrition; 74:607-615.

8) R.R. Vani, U.M. Rawal (1996). Glutathione and glutathione redox cycle in riboflavin supplanted cataractous rat lenses. Indian Journal of pharmacology; 28:93-97.

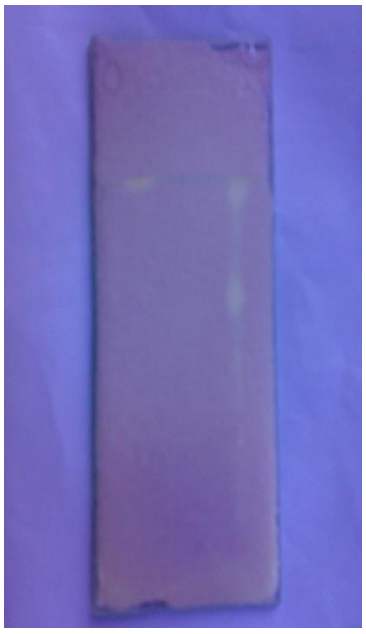
9) Gamal A.Mohamed (2008). Alliuocide G, A new flavonoid with potent α-amylase inhibitory activity from Allium cepa L. ARKIVOC; 11:202-209.

10) Kokate C.K., A.R. Purohit, S.B.Gokhale (2004). Pharmacognosy. 27th edition, Pune, Nirali Prakashan; 106.

11) Shoeb M., Macmanus S.M., Kong-Thoo-Lin p, Celik S., Jaspars M, Nahar L, Sarker S.D. (2007). Bioactivity of the extracts and isolation of lignan and a sesquiterpene from the aerial parts of Centaurea pamphylica (Asteraceae). DARU; 15(3):118-122.

12) Shanmugakumar S.D., Amerjothy S., Balakrishna and Soo-un Kim (2008). Isolation, Characterization and in-vitro evaluation of Kaempferol-3-0-α-1-Rhamanopyranoside from Pithecellobium dulce benth. Against Mycobacterium tuberculosis and its associated secondary infections. Pharmacognosy Magazine. 4(15):559-564.

13) Nikhat F, D.Satynarayana, and Subhramanyam EVS (2009). Isolation, Characterizations and Screening of Antioxidant Activity of the Roots of Syzygiumcuminii (L) Skeel. Asian J. Research Chem.; 2(2):218-221.
14) Naznin Ara and Hasan Nur (2009). In Vitro Antioxidant Activity of Methanolic Leaves and Flowers Extracts of Lippia Alba. Research Journal of Medicine and Medical Sciences; 4(1):107-110.
15) S. Chethan, Shylaja M. Dharmesh, Nagappa G. Malleshi(2008) . Inhibition of aldose reductase from cataracted eye lenses by finger millet (Eleusine coracana) polyphenols. Bioorganic & Medicinal Chemistry; 16:10085–10090
16) Sibel Suzen, Net Das-Evcimen, Pmar Varol, Mutlu Sankaya (2007). Preliminary evaluation of rat kidney aldose inhibitory activity of 2-phenylindole derivatives: affiliation to antioxidant activity. Medicinal Chemistry Research: 16:112-118.
17) G. Manonmani, V.Bhavapriya, S. Kalpana, S.Govindasamy, T.Apparanantham (2005). Antioxidant activity of Cassia fistula (Linn.) flowers in alloxan induced diabetic rats. Journal of Ethnopharmacology; 39-42.
18) Kalyarat Kruawan and Kaew Kangsadalampai (2006). Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs. Thai J. pharm.Sci.; 30:28-35.
19) Suresh Kumar Gupta, Vivekananthan Kalaiselvan, Sushma Shrivastava, Rohit Saxena, Shyam Sunder Agrawal (2009). Inhibitory effect of Trigonella Foenum-Graecum on Galactose Induced Cataracts in a Rat Model; in vitro and in vivo Studies. Journal of Ophthalmic and Vision Research; 4(4):213-219.
20) Marjorie Dunlop (2000). Aldose reductase and the role of the polyol pathway in diabetic nephropathy. Kidney International; 58:1-16



ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, S.Iss 07, 2022

Fig.1 shows the qualitative antioxidant activity of methanolic extract compared with std.quericitin on TLC.

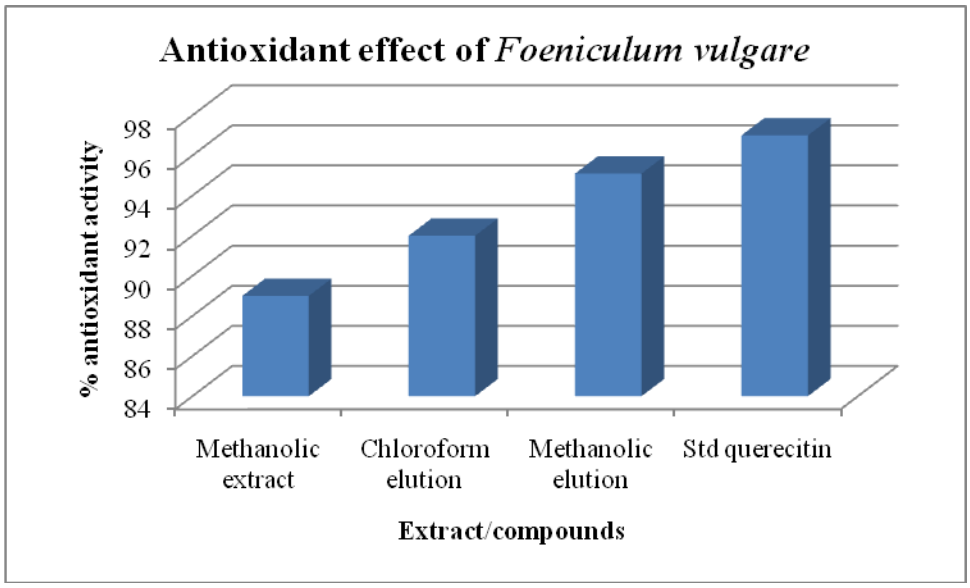
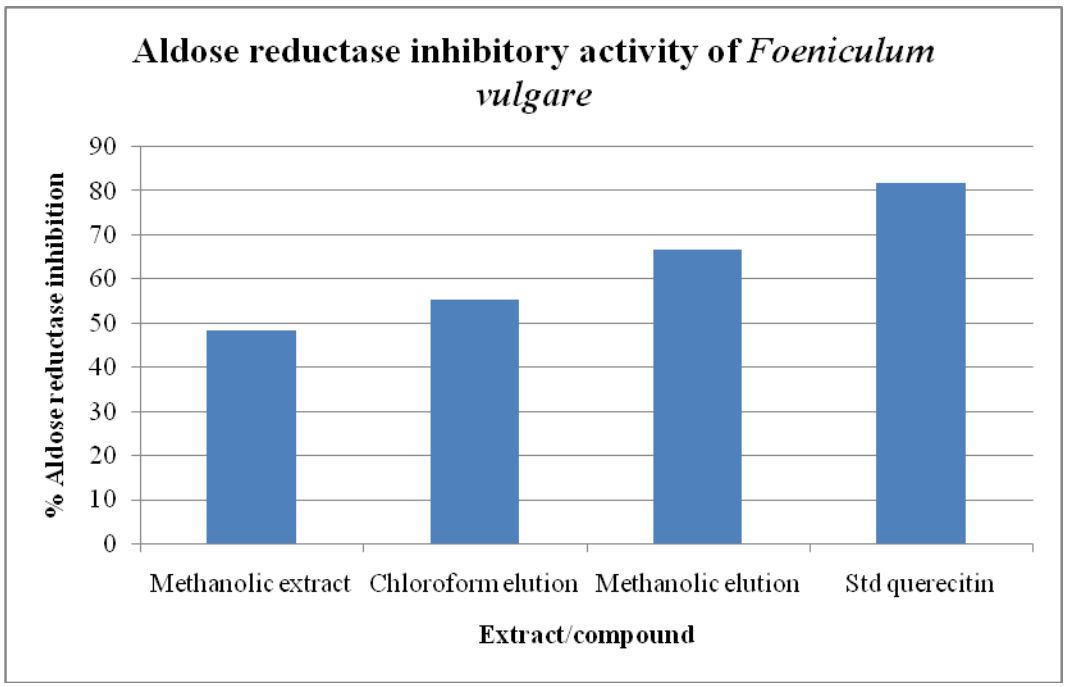


Fig. 2: Shows the % DPPH radical scavenging activity of methanolic extract of Foeniculum vulgare and two isolated fraction compared with std. quericitin.



ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, S.Iss 07, 2022

Fig.3: Shows the % aldose reductase inhibition by methanolic extract of Foeniculum vulgare and two isolated fractions compared with std. quericitin.

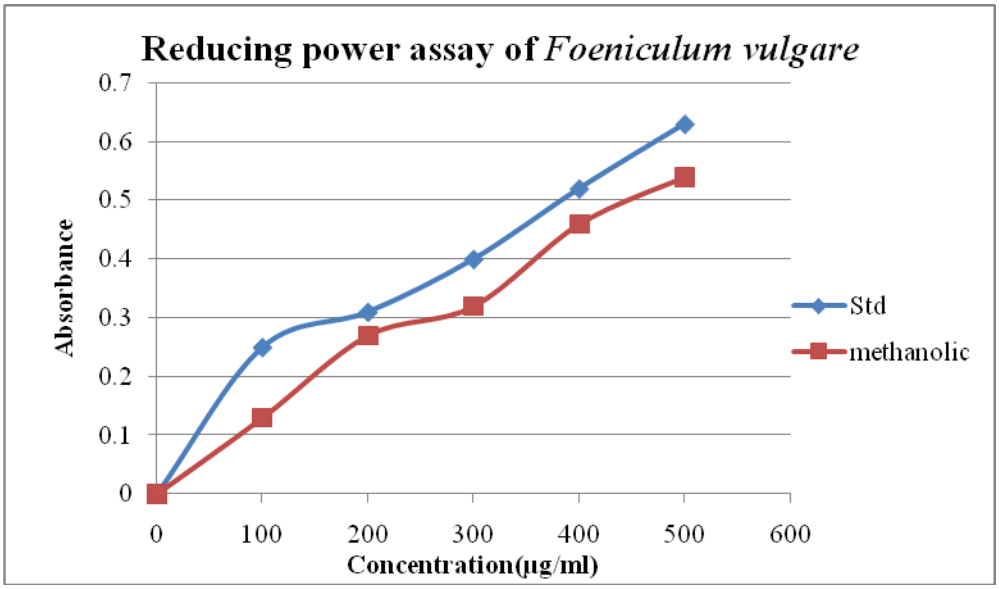


Fig. 4: Shows reducing power of methanolic extract of Foeniculum vulgare as compared to Ascorbic acid.

Table 1. Antioxidant activity (Free radical scavenging activity) & aldose reductase inhibitory activity:-

Compounds/Extracts	Concentration	DPPH radical inhibition (%)	Concentration in µg	% Aldose reductase inhibition
Methanolic extract	10 mg/ml	89±1.0**	100	47.25±0.5**
Methanolic Fraction	20 µg/ml	91.8.±1.2*	50	66.8±1.1**
Chloroform Fraction	20 µg/ml	90.4±0.9**	50	55.23±1.1**
Std querecitin	20 µg/ml	95.8±0.5	50 µg	81.32±1.1

Values are mean±SEM, 3 independent analyses.P<0.05*, P<0.01**as compared to standard (Dunnet's Test)

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, S.Iss 07, 2022

Table 2 shows the reducing power of methanolic extract compared with std. ascorbic acid.

Sr.No.	concentration(PPM)	Std. Ascorbic acid	Methanolic extract of Foeniculum vulgare
1	0	0	0
2	100	0.25	0.13
3	200	0.31	0.27
4	300	0.4	0.32
5	400	0.52	0.46
6	500	0.63	0.54

Note:

Fig.1 shows the qualitative antioxidant activity of methanolic extract compared with std.quericitin on TLC must be printed in colour.