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IN-VITRO ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *OPUNTIA ELATIOR* FRUIT AS WELL AS QUERCETIN

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Abstract: The present study was carried out to evaluate *in-vitro* antioxidant and antidiabetic activity of hydro alcoholic extract of *Opuntia elatior* fruit as well as quercetin. Phytochemical screening of *Opuntia elatior* fruit revealed the presence of carbohydrate, alkaloids, flavonoids, saponin, phenols, sterol, protein and tannins. *In-vitro* antioxidant and antidiabetic activity of hydro alcoholic extract of *Opuntia elatior* fruit as well as quercetin at various concentrations were evaluated by using DPPH inhibition and α-amylase inhibition assay. Hydro-alcoholic extract of *Opuntia elatior* fruit and quercetin showed the antioxidant activity as 38.14 ± 1.07 and 37.74 ± 1.06 % DPPH inhibition, respectively at 200 µg/mL concentration and produced α-amylase inhibition up to 54.68 ± 0.11 and 54.64 ± 0.20 %, respectively, 500 µg/mL concentration. The extract of *Opuntia elatior* fruit as well as quercetin showed good antioxidant and antidiabetic activities in a dose dependent manner respectively.

Keywords: *Opuntia elatior*, Quercetin, Antioxidant, Antidiabetic, DPPH (1, 1-Diphenyl-2-picrylhydrazyl), α-amylase.

Introduction

Free radicals or reactive oxygen species (ROS) are formed in our body as a result of biological oxidation. The over production of free radicals suchas hydroxyl radical, super oxide anion radical, hydrogen peroxide can cause damage to the body and contribute to oxidative stress (Thomson, 1995). Oxidative damage to proteins, DNA and lipid is associated with chronic degenerative diseases including hypertension, coronaryartery disease, cancer, diabetes (Lee *et al*., 2000). The compounds that can scavenge free radicals have great potential in ameliorating such disease processes (Kris-Etherton *et al*., 2002; Di Malteo and Esposito, 2003). Most of active oxygen species are scavenged by endogenous defense systems such as superoxide dismutase, catalase and peroxidase-glutathione system (Rice-Evans and Bourdan, 1993). But endogenous mechanism requires exogenous anti-oxidants from natural sources to scavenge the all free radicals in body.

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Opuntia elatior Mill. is one of species of Opuntiodeae subfamily which is found in Saurashtra region of Gujarat state. Most studies conducted on the *Opuntia elatior* were focused on the chemical composition, lipid fraction, development of seeds, seed oil and polysaccharides, but pharmacological/medicinal properties of this species of *Opuntia* have not been fully explored yet. Quercetin (3,3′,4′,5-7-penta- hydroxyflavone), is an another plant compound (flavonoid) commonly found in many plants and foods. Quercetin (QCT) used for treating conditions of heart and blood vessels including atherosclerosis, high cholesterol, heart disease, and diabetes. The anti-oxidant and antidiabetic activities of extracts of *Opuntia elatior* fruit as well as quercetin has not been evaluated so far. Thus, the present study was carried out to evaluate *in-vitro* antioxidant and antidiabetic properties of hydro-alcoholic extract of *Opuntia elatior* fruit as well as quercetin by DPPH (1,1-Diphenyl-2-picrylhydrazyl) method and by α-amylase assay, respectively.

Materials and methods

Plant materials

Fruits of *Opuntia elatior* plant were collected from local market of Junagadh district and authenticated by Botanist. Quercetin was purchased from the Sigma Aldrich with lot No. SLBD8415V.

Preparation of plant extract

Extract of *Opuntia elatior* fruit was prepared by soxhlet extraction method. About 30 g of powdered material was uniformly packed into a thimble and run in soxhlet extractor. It was exhaustible extracted with methanol for the period of about 72 hours. The extract wasthan filtered with the help of filter paper (Whatman No. 1) and solvent was evaporated in a rotary evaporator to get extract which was used to evaluate *in-vitro* anti-oxidant and antidiabetic activities.

Phytochemical screening of *Opuntia elatior* fruit

Qualitative phytochemical screening of *Opuntia elatior* fruit was carried out for presence of carbohydrate, alkaloids, flavonoids, saponin, phenols, sterol, protein and tannins as per standard methods (Evans, 2002).

Antioxidant activity assays

Anti-oxidant activity of hydro-alcoholic extract of *Opuntia elatior* fruit as well as quercetin was determined using DPPH assay (Itankar *et al*., 2014). Stock solution of hydro-alcoholic plant extract (1 mg/ml) and quercetin dissolved in 1% DMSO were used further. 1 mL of a 100 µM DPPH solution was added to 3 mL of sample solution of different concentrations

(10, 25, 50, 75, 100, 200 µg/mL). Ascorbic acid was used as positive control and prepared in the same manner as above. This method depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine. The determination of the disappearance of free radicals was done using a spectrophotometer. The remaining DPPH which showed maximum absorption at 517nm was measured. The absorbance values were measured at 517 nm and converted into the percentage anti-oxidant activity using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Antidiabetic activity assays

Antidiabetic activity of hydro-alcoholic extract of *Opuntia elatior* fruit as well as quercetin was determined using α-amylase inhibition according to the method of Bernfeld (1955), with minor modifications. Stock solution of hydro-alcoholic plant extract (1 mg/ml) as well as quercetin dissolved in 1% DMSO were used further. Different concentrations (10, 25, 50, 100, 200, 500 µg/mL) of sample solution were prepared from stock solution. Briefly, the sample (500 µL) and the α-amylase (500 µL; 1 unit/mL) were mixed and pre-incubated in 20 mM sodium phosphate buffer (pH 6.9) for 5 min at 37^{o}C. Then, 1 mL of 1% (w/v) starch dissolved in buffer was added to the reaction mixture to make a total volume of 2 mL. The mixture was incubated for 5 min at 37^{o}C. After incubation, 1 mL of dinitrosalicylic acid (DNS) color reagent was added and placed in a boiled bath for 3min and then cooled on ice to room temperature. Further, 6 mL of deionized water was added and α-amylase activity was determined by measuring the absorbance of mixture at 540 nm. Control solution was prepared by adding all the reagents except test or standard. Acarbose was used as a standard for the assay. Per cent inhibition of α-amylase was calculated by using above equation.

Results and discussion

Presence of various phytochemicals upon qualitative phytochemical screening of *Opuntia elatior* fruit extract are shown in table 1 which revealed the presence of carbohydrate, flavonoids, anthocyanin, phenols and protein. The sweet taste of fruit is due to carbohydrate present in the fruit. Most pharmacological activities reported from fruit may due to flavonoids like isorhamnetin, quercetin and kaempferol (Evans, 2002).

Per cent inhibition of DPPH by ascorbic acid, hydroalcoholic extract of *Opuntia elatior* fruit as well as quercetin are tabulated in table 2 and depicted graphically in figure 1. DPPH scavenging activities (%) of hydro-alcoholic extract of cactus fruit as well as quercetin at concentration of 10, 25, 50, 75, 100 and 200 were 11.68 ± 1.65, 28.20 ± 1.35, 29.90 ± 1.29,

34.36 ± 1.07, 36.38 ± 1.14, 38.14 ± 1.07 and 5.24 ± 1.60, 9.46 ± 1.56, 17.71 ± 1.41, 27.56 ± 1.26, 31.38 ± 1.18, 37.74 ± 1.06, respectively. Percent inhibition of DPPH by extract and quercetin were found to increase with increase in concentration. At 200 µg/mL, both quercetin and *Opuntia elatior* extract shown 38.14 ± 1.07 and 37.74 ± 1.06 % inhibition, respectively. The data indicates mild to moderate antioxidant activity of quercetin and *Opuntia elatior* extract. This moderate antioxidant activity of *Opuntia elatior* may be due to presence of betanin and indicaxanthin in it (Chauhan, 2010). Many studies supports our result that *Opuntia ficus-indica* possess anti-oxidant activity (Abd El-Razek and Hassan, 2011; Dib *et al.,* 2014). Quercetin is an abundant dietary flavonoid with well-known radical scavenging properties being often used as a reference compound in many antioxidant tests. Its reaction with 1, 1- diphenyl-2-picrylhydrazyl (artificial DPPH radical) is rapid and stoichiometric (Sak, 2014).

Per cent inhibition of α-amylase by acarbose, hydroalcoholic extract of *Opuntia elatior* fruit as well as quercetin are tabulated in table 3 and depicted graphically in figure 2. Per cent inhibition of α-amylase enzyme activity by extract and quercetin at concentration of 10, 25, 50, 100, 200 and 500 µg/mL of extract of *Opuntia elatior* fruit and quercetin were 29.35 ± 0.23, 32.93 ± 0.15, 37.74 ± 0.09, 40.76 ± 0.27, 41.34 ± 0.08, 54.68 ± 0.11 and 44.81 ± 0.20, 48.03 ± 0.17, 49.02 ± 0.22, 51.48 ± 0.20, 52.21 ± 0.20, 54.64 ± 0.20, respectively. Per cent inhibition of α-amylase enzyme activity by extract and quercetin were found to increase with increase in the concentration. Both *Opuntia elatior* crude extract and quercetin at 500 µg/mL concentration produced 54.68 ± 0.11 and 54.64 ± 0.20 % α-amylase inhibition, respectively. *Opuntia elatior* contains diverse phytochemicals like betalain and betanine. These flavonoids from the plant and quercetin are able to inhibit the α-amylase enzyme *in-vitro.* Basic therapeutic approach with *Opuntia* fruit juice to treat diabetes may be to inhibit the absorption of glucose by retarding the action of gastro-intestinal enzymes such α-amylase. Similar to *Opuntia elatior* in the present study, *Opuntia stricta* fruits extract also shown high level of α-amylase inhibition (Kunyanga *et al*., 2014). Quercetin also found to have a dose-dependent inhibitory effect on α-amylase enzyme (Nickavar and Amin, 2011). Thus, *Opuntia elatior* fruit juice added with quercetin may be good prophylactic or therapeutic approach in many degenerative diseases including diabetes. However, *in-vivo* studies in disease animal model and clinical cases are required to validate the findings of the present study.

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Table 1: Phytochemical screening of hydro-alcoholic extract of *Opuntia elatior* fruit

Chemical test	**Hydro-alcoholic extract of *Opuntia elatior* fruit**
1.Test for carbohydrate	
Molisch's test	+
Benedict test	+
2.Test for alkaloids	
Wagners test	-
Dragandroff test	-
Hagner's test	-
3. Test for flavonoids	
Shinoda test	+
Sulfuric acid test	+
Alkali reagent	+
Lead acetate test	+
4. Test for saponins	
Froth test	-
5. Test for phenols	
$Fecl_3$ test	+
6. Test for sterol	
Salkowasky test	-
7. Test for protein	
Xanthoproteic test	+
8. Test for tannin	
$Fecl_3$ test	-

Table 2: Per cent inhibition of DPPH by ascorbic acid, hydroalcoholic extract of *Opuntia elatior* fruit as well as quercetin

Conc. (µg/mL)	**Ascorbic acid**		***Opuntia elatior* extract**		**Quercetin**	
	Mean Abs.	**%Inhibition**	**Mean Abs.**	**%Inhibition**	**Mean Abs.**	**%Inhibition**
10	0.142	73.78	0.2295	11.68 ± 1.65	0.2463	5.24 ± 1.60
25	0.137	74.59	0.1866	28.20 ± 1.35	0.2353	9.46 ± 1.56
50	0.129	76.14	0.1822	29.90 ± 1.29	0.2139	17.71 ± 1.41
75	0.127	76.47	0.1706	34.36 ± 1.07	0.1883	27.56 ± 1.26
100	0.123	77.28	0.1653	36.38 ± 1.14	0.1783	31.38 ± 1.18
200	0.107	80.12	0.1608	38.14 ± 1.07	0.1618	37.74 ± 1.06
Blank	--	--	0.2600	--	0.2600	--

Abs: Absorbance

Table 3: Per cent inhibition of α-amylase by acarbose, hydroalcoholic extract of *Opuntia elatior* fruit as well as quercetin

Conc. (µg/mL)	Acarbose		*Opuntia elatior* extract		Quercetin	
	Mean Abs.	% Inhibition	Mean Abs.	% Inhibition	Mean Abs.	% Inhibition
10	0.156	37.79	0.1775	29.35 ± 0.23	0.1389	44.81 ± 0.20
25	0.151	39.75	0.1685	32.93 ± 0.15	0.1308	48.03 ± 0.17
50	0.149	40.74	0.1565	37.74 ± 0.09	0.1283	49.02 ± 0.22
100	0.141	43.76	0.1489	40.76 ± 0.27	0.1221	51.48 ± 0.20
200	0.134	46.56	0.1474	41.34 ± 0.08	0.1203	52.21 ± 0.20
500	0.119	52.81	0.1139	54.68 ± 0.11	0.1142	54.64 ± 0.20
Blank	--	--	0.2513	--	0.2513	--

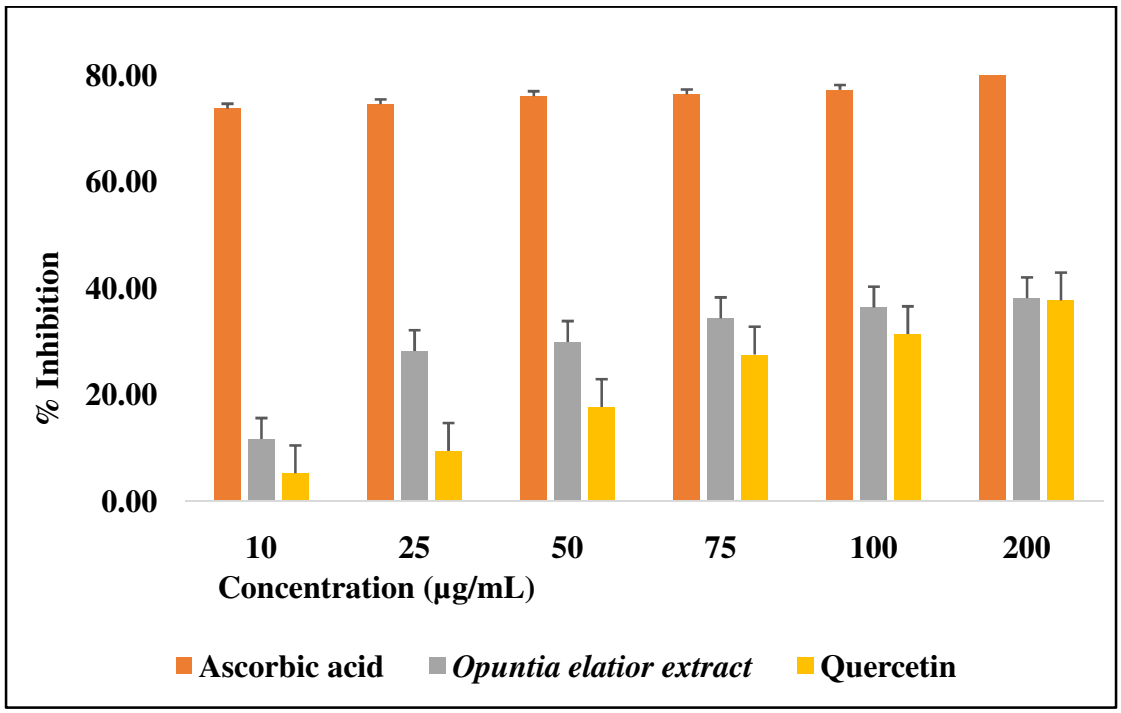

Figure 1: Per cent inhibition of DPPH by ascorbic acid, hydro-alcoholic extract of *Opuntia elatior* fruit as well as quercetin.

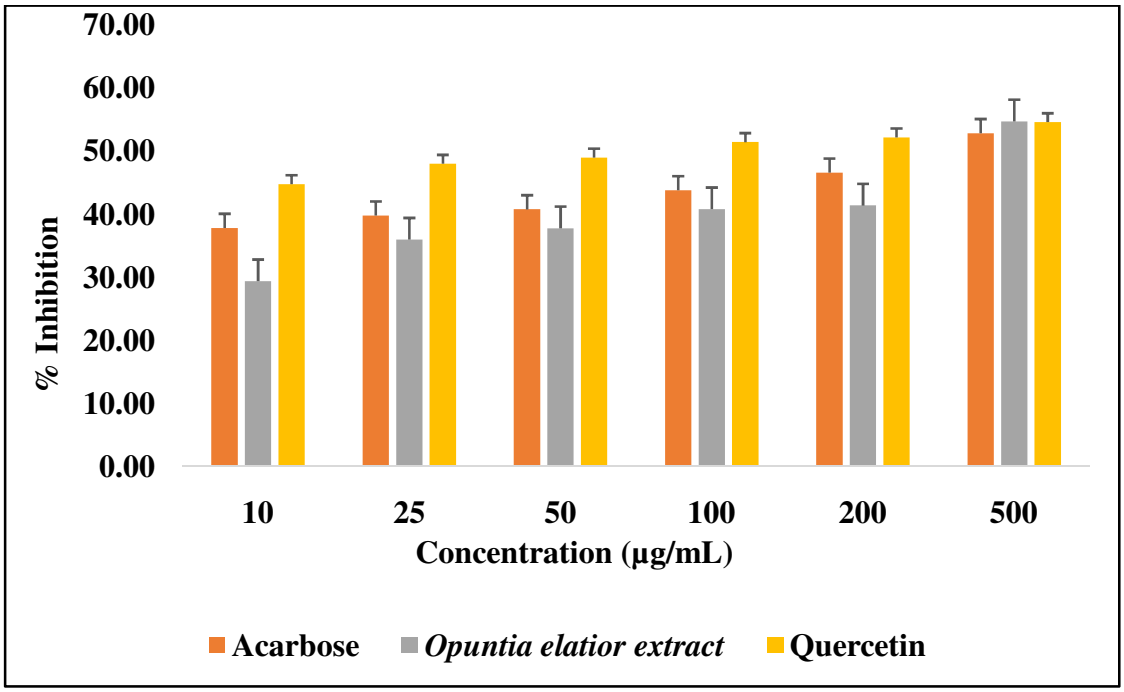

Figure 2: Per cent inhibition of α-amylase by acarbose, hydro-alcoholic extract of *Opuntia elatior* fruit as well as quercetin