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COMPARATIVE EVALUATION OF *IN-VITRO* ANTI-OXIDATIVE AND ANTI-DIABETIC PROPERTIES OF HYDROETHANOLIC EXTRACTS OF PITHECELLOBIUM DULCE AND PROSOPIS CINERARIA LEAF AND FLOWER

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ABSTRACT

In Diabetes Mellitus (DM), elevated level of post prandial hyperglycemia (PPHG) is a major risk factor and its reduction can manage DM. Therapeutic approach of antidiabetic activity is to minimize the production of gastrointestinal glucose and inhibition of carbohydrate digesting α-amylase and α-glucosidase enzymes to control PPHG associated with diabetes. In this study the antidiabetic activity of α – amylase and α – glucosidase enzyme was found to be inhibitory in the leaf and flower extracts of *Pithecellobium dulce* and *Prosopis cineraria*. Antioxidants are helpful in maintaining cellular and systemic health and hence, effectively manage stress developed during diabetes. To determine anti-oxidative activity of the plants, Numerous enzymatic and non-enzymatic parameters like, superoxide dismutase (SOD), catalase (CAT), peroxidase, DPPH, hydroxyl radical scavenching assay and metal chelating activity were tested. Both the plants showed significant potential to manage *invitro* anti-diabetic activities but out of the four extracts, the leaf extract of *P. dulce* showed highest significant inhibitory effect. Moreover, enzyme inhibition was increased with increasing concentration of the extracts. These plants also showed good *invitro* antioxidant activities. All the studied parameters of antioxidant activities in *P. dulce* and *P.cineraria*, significantly increased with increasing concentration of plant extract.

KEYWORDS: *In-vitro* Antidiabetic, *In-vitro* Antioxidative, $IC_{50,}$ *Pithecellobium dulce, Prosopis cinereria* and 50% hydroethanolic extract.

INTRODUCTION

Since the ancient times, herbal plants have been known for their wide use in various streams incorporating medicine, fragrances, beverages, flavours, repellents, cosmetics, nutrition, and other industrial purposes [1]. Traditional plants are known for the presence of large amount of biologically active compounds and for their molecular diversity [2]. They are natural source of plenty of antioxidants which can play a significant action in absorption and neutralization of free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Due to the presence of these antioxidants herbal plants are preferentially evaluated for therapeutic activity to manage the occurrence and mortality rates of number of human diseases [3]. Previously, herbal plant extracts have been reported for the treatment of diabetes as they are having anti-oxidative and anti-diabetic activities to manage the disease [4].

Diabetes Mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, action or both and alters the lipids, carbohydrates and proteins metabolism [5]. It is characterized by Hyperglycemia (high blood sugar) in postprandial and fasting state [6]. Diabetes treatment is complicated by numerous risk factors inherent to the disease, and elevated level of Postprandial Hyperglycemia (PPHG) is one of the major factors [7] .The actions of α-amylase and α-glucosidase enzymes is responsible for the elevation of PPHG. Inhibition of these enzymes plays a major role in managing PPHG in diabetic patients. Inhibition of α-amylase and α-glucosidase enzymes activity leads to a reduction in disaccharides hydrolysis which has beneficial effects on glycemic index control in diabetic patients [8, 9]. Many compounds in plants have been reported for bioactive properties, mainly for the effect of antioxidants which in herbal medicines have the aptitude to scavenge the free radicals, manage carbohydrate metabolism and lowers lipid peroxidation [10]. According to World Health Oraganisation (WHO), traditional plants are used for the treatment of various cardiovascular diseases as they are very effective in treatment with no toxicity and very few side effects.

The plants *Pithecellobium dulce* (jungle jalebi) and *Prosopis cineraria* (Khejri) belong to the family Fabaceae or bean pea family. The plants from this family are largely harvested as crops for human and animal consumption and also for oil, fuel, fertilizers, medicinal, chemicals and horticulture varieties [11]. The whole seed extract of legume has been reported in reducing the high blood sugars in diabetes because of their glycemic index [12]. *P. cineraria* are a moderate sized tree available in various places of India such as Rajasthan,

Uttar Pradesh, Gujarat, Haryana and Tamilnadu and traditionally used for treatment of various ailments like leprosy, leucoderma, dysentery, asthma and earache etc. Pharmacological properties like analgesic, antipyretic, anti-hyperglycemic, antioxidant, antitumor, anti-hypercholesterolemic, nootropic of this plant have been reported from a variety of different plant parts. Various bioactive compounds like flavonoid, alkaloids, diketones, phenolic contents, free amino acids, lipids, b-serotirol, sugars and vitamins have been isolated from various plant parts [13].

P. dulce, is a species of flowering plant. The pods of seeds contain a sweet pulp that can be eaten raw or prepared as a smoothie. It is a thorny tree which can become weedy. It is a tree with multiple uses; food (sweet pods), firewood, honey, fodder, soap oil, tannin, hedges are obtained from this plant and it can also survive hostile climates. This plant is known to possess many medicinal properties such as antimicrobial, astringent, dysentery, dermatitis [14], anti-inflammation [15], emollient, abortifiacient and antidiabetic properties [16] and antioxidant properties [17]. The aim of the present study is to compare the *in-vitro* antidiabetic and antioxidative activities of *P. cineraria* and *P. dulce.*

MATERIAL AND METHODS

Chemicals

All chemical brought in use for the study were of analytical grade and were purchased from HIMEDIA (India), SRL (India), CDH (India), Qualigens (India/Germany), Sigma (USA).

Collection and Preparation of Plant Extract

The leaf and flowers of *P. dulce* were collected from, Jaipur nursary, Rajasthan whereas leaves and flower were harvested from local identified *P. cineraria*. The plant parts were cabinet dried and were subjected to size reduction to a coarse powder using grinder and passed through sieve no. 80 to get a powder of uniform particle size. The powder so obtained were packed into soxhlet apparatus and extracted with 50% ethanol till solution in the thimble becomes clear and was concentrated to dryness under reduced pressure at 60±1°C in rotatory vacuum evaporator. The extract were dried at 40-45°C in hot air oven till solid to semisolid mass were obtained and stored in an air-tight container in refrigerator below 10°C [18]. The suspension of the hydro-ethanolic extract was used on each day of the experiment.

Assays for *Invitro* Antidiabetic Activity

α-Amylase Enzyme Inhibition Assay

α- amylase activity was measured by using the DNS method. To 500 µL of plant extract500 µL of 0.02 M sodium phosphate buffer with 6 mM sodium chloride and 0.04 units of α-amylase solution were added. The reaction mixture was then incubated for 10 min at 37°C. After this, 500 µL of 1% starch solution dissolved in 0.02 M sodium phosphate buffer was added. 1.0 ml of 3, 5 dNSA reagents then stopped the reaction. The test tubes were then incubated in a boiling bath water for 5 min and cooled to room temperature. The absorbance at 540 nm was measured after diluting the reaction mixture by adding 10 ml distilled water [19]. Similarly, control samples were prepared, but without plant extracts and were compared with test samples containing. The results were expressed as calculated using the formula:

I= (Abs control – Abs sample) / Abs control

α – Glucosidase Enzyme Inhibition Assay

1 ml solution of starch substrate (2 % w/v maltose or sucrose) was incubated with 0.2 M Tris buffer pH 8.0 and the plant extract at 37°C for 5 min. 1 ml of alpha-glucosidase enzyme (1U/ml) was added to this to start the reaction, the test tubes were incubated for 40 min at 35°C thereafter.

2 ml of 6N HCl was then added to stop the reaction. The absorbance was then measured at 540nm to estimate the intensity of the colour [20]. The activity was estimated by calculating % inhibition using the same formula as above.

Methods Employed For *In-Vitro* Anti-Oxidant Activity

All extracts were analyzed for antioxidant potential by the following methods:

***In-Vitro* Assays for Enzymatic Antioxidants**

Superoxide Dismutase

1 ml of 125 mM sodium carbonate, 0.4 ml of 25 µM NBT and 0.2 ml of 0.1 mM EDTA were added to 0.5 ml of plant extract. 0.4 ml of 1 mM Hydroxylamine hydrochloride was added to initiate the reaction after which absorbance was taken at 5 min intervals at 560 nm. SOD activity was expressed as the amount of enzyme required for inhibiting the reduction of NBT by 50% [21] .The specific activity was expressed in terms of units per mg of protein.

Catalase

Titrimetric method was used to determine the Catalase activity [22]. 5 ml of 300 µM phosphate buffer (pH 6.8) containing 100 µM hydrogen peroxide (H_2O_2) was added to 1ml plant extract and the reaction mixture was left at 25°C for 1 min. 10 ml of 2% sulphuric acid was then added to arrest the reaction. The residual H_2O_2 was titrated against 0.01N potassium permanganate till pink colour was obtained. Enzyme activity was estimated by calculating the decomposition of µM H_2O_2 per min per mg protein.

Peroxidase

Peroxidase activity was estimated by adding3.5 ml of phosphate buffer (pH 6.5) and 0.1 ml of O- dianisidine solution to 0.2 ml of plant extract. 0.2 ml of 0.2 mM H_2O_2 was added to initiate the reaction and absorbance was then measured every 30sec intervals upto 3 min. The extinction coefficient of oxidized O-dianisidine was calculated for estimating peroxidase activity. The enzyme activity was expressed as units per mg of protein [23].

***In-Vitro* Assay of Non -Enzymatic Antioxidants**

DPPH Radical Scavenging Assay

3 ml of the plant extract was added to 1ml of 0.1 mM solution of DPPH made in methanol. This was incubated at 37°C. After 30 min, its absorbance was measured against control (Ascorbic acid and BHT) using a spectrophotometer (Hitachi) at 517 nm. The absorbance values of the test samples were compared with those of the controls (without extract) to calculate the percentage inhibition [24] The radical scavenging activity was then estimated by calculating the inhibition percentage (I) as follows.

I= (Abs control – Abs sample) / Abs control X 100

Metal Chelating Activity

To a solution of 1 ml of ferrous sulphate (0.125 mM), 1 ml of the extract was added. 1 ml of ferrozine (0.3125 mM) was then added to initiate the reaction. After this the reaction mix was incubated at room temperature for 10 min. Thereafter, absorbance was measured at 562 nm, while EDTA or Citric acid was used as positive control [25]. The ability of sample to chelate ferrous was calculated relative to the control using formula.

Chelating effect = (Abs control – Abs sample) / Abs control

Hydroxyl Radical Scavenging Assay

The competition between hydroxyl radicals generated from deoxyribose and the extract was measured from Fe3+/ascorbate/EDTA/H_2O_2 system [26]. A reaction mixture was prepared with 3.0 mM deoxyribose, 0.1 mM $FeCI_3$, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2 and 20 mM phosphate buffer (pH 7.4) and 0.1ml of extract was added to it making a final volume of 3.0 ml. Reactions were carried out in triplicate. These were incubated for 1 hr at 37ºC after which 1 ml of thiobarbituric acid (1%) and 1.0 ml trichloroacetic acid (2.8%) were added and then incubated for another 20 min at 100ºC. The test tubes were cooled and the absorbance of the reaction mixture was measured at532 nm. Deoxyribose and buffer were taken as blank. Inhibition percent (I) of deoxyribose degradation was calculated using the formula.

I= (Abs control – Abs sample) / Abs control.

Ascorbic acid was used as a positive control.

Calculating IC_{50} Values

The antiradical and antidiabetic activity of tested compounds was worked out by calculating IC_{50} (concentration of a compound inhibiting the activity of a test solution by 50%) using MS Excel.

Statistical Analysis

Results are expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc multiple comparison test using SPSS (version 16.0) and student's't'-test using Sigma Plot (version 8.0). The values of $P<0.05$ were considered as statistically significant.

RESEULT AND DISCUSSION

***In-Vitro* Antidiabetic Properties of P. Dulce and P. Cineraria**

DM is a chronic disease, because of the several risk factor inherited to the disease the treatment of DM is complicated. Current progress of the activity of α-amylase and α -glucosidase intestinal enzymes are essential in carbohydrate digestion and glucose absorption, have shown the way to go forward of newer pharmacological agents. Elevated level of these enzymes has lead to the development of Postprandial Hyperglycemia (PPHG) in diabetes. PPHG is directly associated with micro and macro vascular complications of diabetes which are more strongly connected with cardiovascular diseases than fasting blood

glucose. It was proposed that inhibition of these digestive enzymes would hinder starch degradation and oligosaccharides, which would in turn reduce glucose absorption and as a result of it the elevated level of PPHG reduced [27]. The inhibitor of α- glucosidase enzyme retards carbohydrate digestion and slows down the absorption of glucose. The standard of alpha glucosidase, Acarbose reduces absoption of starch and diasaccharides [28]. Inhibition of α- amylase and α- glucosidase is one of the thereupatical approaches to reduce the PPHG in diabetes [29]. Synthetic inhibitors of these enzymes, Acarbose causes side effects such as abdominal pain, diarrhea and soft faeces in the colon.

Result of this study shows proficient inhibition of α–amylase enzyme in leaf and flower extracts of *P. dulce* and *P. cineraria*. The inhibitory reaction mechanism of α-amylase enzyme by both the plants protein inhibitor is not evidently implicit. Hence, it was assumed that the *P. dulce* and *P. cineraria* might cause the conformational changes about the inhibitory effect of intestinal enzymes as they were chemical investigated for flavonoid, alkaloids, diketones, phenolic contents, free amino acids, lipids, b-serotirol, sugars and vitamins in *P. cineraria* [13] and for tannins, flavonoids, glycosides, alkaloids, saponins, lipids, phospholipids, steroids and fatty acids in *P. dulce* [30]. The percent inhibition at 200, 400, 600, 800 and 1000µg/ml concentration showed a concentration dependent reduction in percentage inhibition. Out of these two plants the leaf of *P. dulce* was found to be more efficient with IC_{50} value of 570µg/ml as compared to the flower of *P.dulce* of IC_{50} value 624µg/ml and of leaf and flower extract of *P.cineraria* with IC_{50} values 598µg/ml and 690µg/ml respectively. The percent inhibition of all the extracts of the two plants was also compared to the standard drug Acarbose having the IC_{50} values of 502µg/ml.

Similarly, when activity of α-glucosidase was measured to evaluate antidiabetic activity, in all the experiments it was found to be inhibitory. The hydro-ethanolic leaf extract of *P.dulce* showed more efficient reduction with IC_{50} value of 545µg/ml as compared to the flower of *P. dulce* of IC_{50} value 612µg/ml and as of leaf and flower of *P.cineraria* with IC_{50} values 678µg/ml and 594µg/ml respectively. The result of this is also compared to Acarbose, a standard drug used for α- glucosidase having IC_{50} value of 422.73µg/ml.

Table 1(a): % inhibition and IC_{50} values of hydro-ethanolic leaf and flower extract of *P. dulce* and *P. cineraria* that actively inhibits α- amylase and α- glucosidase enzymes. The data are expressed as mean ± Standard Deviation of triplicate readings (n=3)

Sample	Concentration (µg/ml)	α - glucosidase		α- amylase	
		% Inhibition (± SD)	IC_{50}	% Inhibition (± SD	IC_{50}
PD leaf	200	21.05 ± 3.17	545	20.01 ± 2.73	570
	400	33.06 ± 6.68		40.14 ± 15.04	
	600	57.93 ± 7.89		66.28 ± 4.12	
	800	73.45 ± 9.06		79.24 ± 0.69	
	1000	91.67 ± 11.43		93.64 ± 1.74	
PD Flower	200	16.11 ± 1.01	612	18.10 ± 1.2	624
	400	27.63 ± 2.75		27.11 ± 2.08	
	600	49.44 ± 5.72		47.23 ± 9.01	
	800	64.29 ± 15.14		62.01 ± 11.01	
	1000	85.71 ± 9.08		84.27 ± 3.17	
PC Leaf	200	14.23 ± 17.01	678	19.82 ± 11.02	598
	400	30.71 ± 12.81		32.63 ± 9.18	
	600	45.24 ± 7.08		64.77 ± 5.04	
	800	68.04 ± 5.44		79.11 ± 15.19	
	1000	81.63 ± 3.29		90.17 ± 8.23	
PC Flower	200	16.76 ± 2.02	594	15.28 ± 13.19	690
	400	38.08 ± 9.86		28.57 ± 9.24	
	600	54.44 ±7.32		45.12 ± 2.17	
	800	65.12 ± 12.03		67.81 ± 1.27	
	1000	77.0 ± 9.32		81.72 ± 0.73	

Table 1 (b): % inhibition and IC_{50} values of standards drug Acarbose for α-amylase and α-glucosidase. The data are expressed as mean ± Standard Deviation of triplicate readings (n=3).

Acarbose (α-amylase)		Acarbose (α-glucosidase)	
Conc. (µg/ml)	% Inhibition	Conc. (µg/ml)	% Inhibition
200	32.3±6.929	0.2	41.0±1.087
400	47.0±3.323	0.4	49.47±1.12
600	56.38±1.202	0.6	68.9±0.73
800	69.95±0.565	0.8	74.43±1.23
1000	85.265±1.279	1	93.2±0.43
IC 50 (µg/ml)	**502**	IC 50 (µg/ml)	**422.73 ± 7.89**

In-Vitro* Antioxidant Potential of Leaf and Flower Extracts of *P. Dulce* and *P. Cineraria.

Oxidative Stress generated free radicals can damage various metabolic pathways. The biological damage caused by free radicals is lipid peroxidation [31]. Free radicals damage is probably involved in the destruction of beta cell and in the pathogenesis of diabetes mellitus. Bioactive compounds of plants contribute to their antioxidant potential. The majority of

natural antioxidants are found in bark, fruit, flower, leaf, pod, and seed of plants [32]. Most of these compounds are phenolic or polyphenolic in nature example tocopherols, flavonoids, and derivatives of cinnamic acid and other organic acids.

Enzymatic Antioxidants

Enzymatic antioxidants are Super Oxide Dismutase (SOD), Catalase (CAT) and Peroxidase and they differ from each other in structure, tissue availability and requirement of cofactors. SOD enzyme demolishes the superoxide radical and can create hydrogen peroxide, which has high toxic properties [33]. It has been reported as a major antioxidant defence enzyme that scavenge superoxide anion by converting to hydrogen peroxide thus reduce the toxic consequence caused by this radical [34]. Catalase, an another antioxidant enzyme which plays an important function in the body defense mechanism against the destructive property of the reactive oxygen species (ROS) and free radicals in biological systems [35]. Catalase catalyzes the dismutation of hydrogen peroxide in water and oxygen [36]. Peroxidase reduces H_2O_2 to water while oxidizing a variety of substrates. Thus, peroxidases are oxidoreductases which use H_2O_2 as electron acceptor for catalyzing different oxidative reactions [34].

The level of enzymatic antioxidants such as SOD, CAT and Peroxidase of leaf and flower extract of *P. dulce* and *P. cineraria* showed in the table 2. In case of enzyme activity of hydro-ethanolic leaf and flower extracts *P. dulce* and *P. cineraria* showed significant inhibitory effect, increased with increasing concentration of plant extracts. From both the plant the SOD, CAT and Peroxidase levels of leaf extract of *P. dulce* showed higher enzyme inhibition from 2.89 ± 3.6 at 200 µg/ml to 16.79 ± 9.23 at 1000 µg/ml in case of SOD, from 4.82 ± 2.1 at 200 µg/ml to 14.89 ± 5.2 at 1000 µg/ml in case of Catalase and from 4.7 ± 1.8 at 200 µg/ml to 12.13 ± 2.6 at1000 µg/ml in case of peroxide as compared to the flower of *P. dulce* with maximum inhibition at higher concentration of 1000 µg/ml of 13.71 ± 1.89, 13.73 ± 0.9 and 11.99 ± 1.6 respectiviely in SOD, Catalase and Peroxidase. Hence, leaf of *P. dulce* shows efficient inhibitory action as compared to its flower as well as to the leaf and flower of *P. cineraria* the leaf and flower of *P. cineraria* as shown in the Table 2.

Table 2: Specific enzyme activity of enzymatic antioxidants of leaf and flower extracts of *P. dulce* and *P. cineraria*. The data are expressed as mean ± Standard Deviation of triplicate readings (n=3)

Sample	Conc (µg/ml)	Specific activity of enzymatic antioxidants (µ/mg)		
		SOD	Catalase	Peroxidase
PD Leaf	200	2.89 ± 3.6	4.82 ± 2.1	4.7 ± 1.8
	400	6.13 ± 11.18	6.91 ± 2.8	7.2 ± 2.9
	600	12.63 ± 5.19	10.19 ± 3.8	9.27 ± 1.9
	800	14.24 ± 3.18	12.01 ± 4.2	11.72 ± 1.4
	1000	16.79 ± 9.23	14.89 ± 5.2	12.13 ± 2.6
PD Flower	200	2.52 ± 1.73	4.12± 1.8	3.9 ± 3.1
	400	5.24 ± 12.63	7.28 ± 1.4	6.8 ± 1.3
	600	8.03 ± 4.12	8.12 ± 2.8	8.23 ± 4.7
	800	10.38 ± 11.26	11.63 ± 4.2	10.22 ± 4.2
	1000	13.71 ± 1.89	13.73 ± 0.9	11.99 ± 1.6
PC Leaf	200	2.68 ± 1.67	2.41 ± 7.1	4.0 ± 2.8
	400	6.08 ± 11.09	4.92 ± 5.3	6.9 ± 1.8
	600	8.15 ± 5.06	6.72 ± 3.1	8.7 ± 1.4
	800	11.09 ± 3.72	8.12 ± 1.8	10.4 ± 2.3
	1000	12.74 ± 1.04	11.03 ± 2.1	11.9 ± 5.4
PC Flower	200	2.13 ± 6.99	3.91 ± 0.91	3.4 ± 3.2
	400	6.23 ± 1.08	5.98 ± 1.7	5.3 ±2.02
	600	8.12 ± 2.31	7.41 ± 7.5	6.9 ± 1.10
	800	10.71 ± 5.20	9.81 ± 2.3	7.6 ± 2.8
	1000	11.23 ± 3.08	11.8 ± 2.7	10.4 ± 2.4

Non-Enzymatic Antioxidants

DPPH accepts electron and hydrogen radical to become a stable molecule. The capability of natural antioxidants to reduce the DPPH free radical is measured by decrease in absorbance at 517nm and compared with the positive control Butylated Hydroxyl Toluene (BHT) used for DPPH. The free radical scavenging activity of extracts is shown in the table 3(a). Both the plants showed efficient inhibitory activity, increased with increasing concentration of plant extracts. The highest inhibitory activity shown by the leaf extract of *P. dulce* with IC_{50} value of 434µg/ml and rest of the other extracts such as flower of *P. dulce* having IC_{50} value of 467µg/ml, *P. cineraria* Leaf and flower having IC_{50} values 503µg/ml and 590µg/ml repectively which is lower as that of leaf of *P. dulce*. The IC_{50} value of positive control for DPPH is 0.625mg/ml. When compared to the positive control the entire extracts showed better efficacy at the level of IC_{50} values. This difference of percent inihibition at five different concentration and IC_{50} values of each extract could be because of the variation in

secondary metabolite present in both the plants and due to this the level of natural antioxidants are different in both the plants.

Table 3 (a): % inhibition and IC_{50} values of hydro-ethanolic leaf and flower extract of *P. dulce* and *P. cineraria* that actively showed antioxidant activity of DPPH. The data are expressed as mean ± Standard Deviation of triplicate readings (n=3)

Sample	Concentration (µg/ml)	% inhibition (± SD)	IC_{50} (µg/ml)
PD Leaf	200	29.6 ± 5.8	434
	400	46.4 ± 7.4	
	600	72.1 ± 1.72	
	800	99.2 ± 2.01	
	1000	101.3 ± 8.3	
PD Flower	200	28.1 ± 1.03	467
	400	45.2 ± 0.99	
	600	58.3 ± 2.05	
	800	72.4 ± 1.01	
	1000	97.5 ± 4.11	
PC Leaf	200	20.1 ± 5.01	503
	400	43.2 ± 4.12	
	600	68.4 ± 2.13	
	800	84.3 ± 1.13	
	1000	108.1 ± 3.1	
PC Flower	200	23.2 ± 8.1	590
	400	32.8 ± 2.3	
	600	51.1 ± 6.3	
	800	82.3 ± 4.4	
	1000	93.4 ± 1.0	

Metal Chelating Activity

Normal concentration of metals has important roles in the body metabolism such as oxygen transport respiration and activity of many enzymes. The most reactive metal is iron and catalyzes the oxidative changes in lipid, protein and other components [37]. Chelating agent treatment is a most favorable process to decrease metals toxicity in organisms. The metal chelating activity of hydro-ethanolic extracts of both the plants was found to possess concentration dependent inhibitory effects. The IC_{50} values of leaf and flower extracts of *P. dulce* were found 381 µg/ml and 534µg/ml respectively and the IC_{50} of *P. cineraria* were found 594µg/m in leaf and 410µg/ml in flower extracts. The IC_{50} of EDTA were found 162.82µg/ml. the results reveal that the leaf of *P. dulce* are good to manage metal toxicity as compared to the flower of it and as they are compared to leaf and flower of P. cineraria they were found good to manage metal chelating activity.

Table 3 (b): % inhibition and IC_{50} values of hydro-ethanolic leaf and flower extract of *P. dulce* and *P. cineraria* that actively shows antioxidant activity of Metal Chelating Activity. The data are expressed as mean ± Standard Deviation of triplicate readings (n=3)

Sample	Concentration (µg/ml)	% inhibition (± SD)	IC_{50}
PD Leaf	200	24.9 ± 1.30	381
	400	39.01 ± 0.89	
	600	54.6 ± 2.1	
	800	70.1 ± 0.99	
	1000	85.01 ± 1.01	
PD Flower	200	17.01 ± 3.2	720
	400	30.14 ± 1.1	
	600	56.76 ± 1.01	
	800	74.89 ± 1.04	
	1000	90.61 ± 0.76	
PC Leaf	200	19.21 ± 4.3	587
	400	35.41 ± 1.8	
	600	51.22 ± 1.2	
	800	77.18 ± 0.96	
	1000	89.72 ± 1.01	
PC Flower	200	27.8 ± 1.0	410
	400	49.2 ± 0.84	
	600	65.4 ± 1.01	
	800	87.1 ± 0.79	
	1000	105.03 ± 0.69	

Hydroxyl Radical Scavenging Assay

Hydroxyl radicals are the most reactive radicals among the reactive oxygen species. During aerobic metabolism, hydroxyl radicals are endogenously generated. A single hydroxyl radical may lead to cell death, as they produces number of lipid hydroperoxides and these molecules severely disrupts the cell membrane [38]. The hydro-ethanolic leaf and flower extracts of *P. dulce* and *P. cineraria* were found to possess concentration dependent scavenging activity on hydroxyl radicals and the results were given in the table 3 (c). The IC_{50} values for hydro-ethanolic leaf and flower extracts of *P. dulce* were found to be 298 µg/ml and324 µg/ml and leaf and flower of *P. cineraria* were found to be 320µg/ml and 408µg/ml respectively. The IC_{50} of ascorbic acid were found to be 168.3µg/ml. This reveals that the leaf of *P. dulce* shows higher inhibitory activity against hydroxyl radicals as compared to the flower of it and as that of both the extracts of *P. cineraria*.

Table 3 (c): % inhibition and IC_{50} values of hydro-ethanolic leaf and flower extract of *P. dulce* and *P. cineraria* that actively shows antioxidant activity of Hydroxyl Radical Scavenging Assay. The data are expressed as mean ± Standard Deviation of triplicate readings (n=3)

Sample	Conc. (µg/ml)	% inhibition (± SD)	IC50 (µg/ml)
PD Leaf	200	27 ± 1.11	298
	400	71 ± 2.08	
	600	108 ± 0.98	
	800	150 ± 1.98	
	1000	198 ± 3.23	
PD Flower	200	21 ± 3.23	324
	400	59 ± 2.11	
	600	89 ± 1.14	
	800	136 ± 5.23	
	1000	176 ± 1.18	
PC Leaf	200	23 ± 2.81	320
	400	65 ± 1.08	
	600	84 ± 2.33	
	800	124 ± 3.81	
	1000	146 ± 2.11	
PC Flower	200	25 ± 0.69	408
	400	48 ± 1.23	
	600	71 ± 0.89	
	800	114 ± 2.01	
	1000	139 ± 1.41	

Table 3(d): % inhibition and IC_{50} values of standards (Ascorbic acid and EDTA) for non-enzymatic antioxidants DPPH, Hydroxyl Radical Scavenging Activity and Metal Chelating Activity. The data are expressed as mean ± Standard Deviation of triplicate readings (n=3)

Conc. (mg/ml)	% Inhibition of Various Controls		
	Ascorbic acid (DPPH)	Ascorbic Acid (Hydroxyl Radical Scavenging)	EDTA (Metal Chelating Activity)
0.2	6.87 ± 3.88	64.2 ± 4.73	60.68 ± 0.02
0.4	21.33 ± 2.34	70.4 ± 2.34	69.90 ± 0.08
0.6	46.78 ± 7.8	76.01 ± 1.59	83.78 ± 0.01
0.8	87.3 ± 8.78	82.63 ± 3.07	95.90 ± 0.07
1.0	130.4 ± 10.18	88.87 ± 2.78	104.87 ± 0.02
IC 50 (mg/ml)	**0.625**	**0.168.3**	**0.163**

CONCLUSION

From the present study we verdict extremely encouraging outcome for the management of Diabetes Mellitus in *in-vitro* assessment. This study shows that the hydro-ethanolic leaf and flower extracts of *P. dulce* and *P. cineraria* have good potential to manage DM as well as oxidative stress generated by it. However, these abilities were at highest in the leaf extract of *P. dulce* which could be used as a good source for antidiabetic drugs. Based on the escort obtained from *in-vitro* studies, *in-vivo* experimentation can be planned for further authentication of the obtained results.

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